Volume LXXIV Number 2

THE BOTANICAL GAZETTE

Editor: JOHN M. COULTER

OCTOBER 1922

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The University of Chicago Press
CHICAGO, ILLINOIS, U.S.A.

THE CAMBRIDGE UNIVERSITY PRESS, *London*
THE MARUZEN-KABUSHIKI-KAISHA, *Tokyo, Osaka, Kyoto, Fukuoka, Sendai*
THE MISSION BOOK COMPANY, *Shanghai*

30 OCT 1922 T. KN

Volume LXXIV Number 2

THE BOTANICAL GAZETTE

A MONTHLY JOURNAL EMBRACING ALL DEPARTMENTS OF BOTANICAL SCIENCE

EDITED BY

JOHN M. COULTER

With the assistance of other members of the botanical staff of the University of Chicago

Issued October 16, 1922

The Botanical Gazette is published in eight issues annually (omitting January and February, and July and August) by the University of Chicago at the University Press, 5750 Ellis Avenue, Chicago, Ill. ¶The subscription price is $9.00 per year; the price of single copies is $1.25. Orders for service of less than a half-year will be charged at the single-copy rate. ¶Postage is prepaid by the publishers on all orders from the United States, Mexico, Cuba, Porto Rico, Panama Canal Zone, Republic of Panama, Dominican Republic, El Salvador, Bolivia, Colombia, Honduras, Nicaragua, Peru, Hawaiian Islands, Philippine Islands, Guam, Samoan Islands, Shanghai. ¶Postage is charged extra as follows: For Canada, 30 cents on annual subscriptions (total $9.30); on single copies 4 cents (total $1.29); for all other countries in the Postal Union, 60 cents on annual subscriptions (total $9.60), on single copies 8 cents (total $1.33). ¶Patrons are requested to make all remittances payable to The University of Chicago Press in postal or express money orders or bank drafts.

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Claims for missing numbers should be made within the month following the regular month of publication. The publishers expect to supply missing numbers free only when losses have been sustained in transit and when the reserve stock will permit.

Business correspondence should be addressed to The University of Chicago Press, Chicago, Ill.

Communications for the editor and manuscripts should be addressed to the Editor of the BOTANICAL GAZETTE, The University of Chicago, Chicago, Ill.

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Entered as second-class matter August 21, 1896, at the Post-Office at Chicago, Illinois, under the Act of March 3, 1879.

Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized on July 15, 1918.

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VOLUME LXXIV NUMBER 2

THE
BOTANICAL GAZETTE

October 1922

HETEROTHECA GRIEVII THE MICROSPORANGE OF HETERANGIUM GRIEVII

MARGARET BENSON

(WITH PLATES IV, V, AND EIGHT FIGURES)

Introduction

The sporangia here described have so far only been recorded from the Pettycur deposits of the Calciferous Sandstone Series of Scotland. They have been found in three blocks, one of which, presented to the writer by Professor BAYLEY BALFOUR in 1905, yielded also some of the specimens of *Sphaerostoma* of which a description has already been published (**5**); the other two blocks were collected at Pettycur by the writer in 1910. In all three blocks they were found associated with *Heterangium Grievii*. Their somewhat flattened fusiform shape is approximately that of *Telangium Scottii* (**4**), but the dimensions are less. *T. Scottii* measures about 2×3 mm. in width at its maximum girth and 4 mm. in length, whereas the new sporange only measures roughly 1×2 mm. in width and 3.5–4 mm. in length. A further resemblance to *Telangium* is their possession of longitudinal loculi or series of loculi, and of peripherally placed tracheids. These resemblances do not seem adequate to suggest its inclusion in this form genus, because there are many remarkably distinctive characters in the new form.

In the first place, each peripheral loculus of *Telangium* is represented in *Heterotheca* by a series of relatively small loculi.

Secondly, central loculi as well as peripheral ones occur in the new form. A third outstanding difference is the absence of any evidence that the sporange underwent apical dehiscence, which is almost universally the case in species of *Telangium*, whether Lower Carboniferous (for example, *T. affine*) or Upper Carboniferous (for example, *T. Scottii*, etc.). The only exception known to me is a species[1] described by NATHORST (11). A fourth outstanding difference from *Telangium Scottii* (the only species so far described with structure preserved) is the presence in the new form of water storage tracheids, which, although few in the normal specimens, are very abundant in the partially sterile specimens. Lastly, a character is found which at first sight seems of minor importance. The new sporange retains the twofold cortical sclerotic plates which form so characteristic a feature of the cortex of *Heterangium* stem and petiole. It is this latter feature which the writer regards as irrefutable evidence of the safe attribution of these microsporangia to *Heterangium Grievii*, with which they have invariably been found associated. Although, therefore, the sporange has the same claim to the name of the parent plant as either stem or petiole, it has been found convenient to refer to it under the name of **Heterotheca Grievii.**

DIAGNOSIS.—I have founded the form genus *Heterotheca* for the reception of such microsporangia as resemble *Telangium* in form and possibly in insertion, but possess vertical sclerotic plates in their periphery and horizontal sclerotic plates deeper down in the cortex, like the vegetative organs of *Heterangium Grievii*. The loculi are not confined to the periphery, but central ones also occur.

The specific name of the form now described is that of the parent plant.

Form, anatomical structure, and grouping of specimens

FORM.—The form of the sporange can be deduced from the various series of sections through single specimens. Some of these have been plotted out to scale in text figs. 1 and 2. These show

[1] This species, of which the type specimen was figured by ARBER (1) under the name of *Carpolithus Nathorstii*, may prove to be a species of *Telangium*, owing to NATHORST'S discovery of its longitudinal loculi filled with microspores. The plate referred to exhibits what was possibly approximately the habit of *Heterotheca Grievii*.

H. Grievii to have had a tapering apex and a maximum girth nearer the base than the apex. The majority of the transverse sections through the upper part are circular, but in the basal region the sectional area is oval (figs. 8, 9), hence there are two aspects of each sporange given in text figs. 1 and 2.

In longitudinal sections the apical part is seen in surface view if present, as indicated in text fig. 3. In the upper third, the surface loculi are only slightly rubbed down in the center, the lateral ones being seen almost in surface view. If two sections are secured

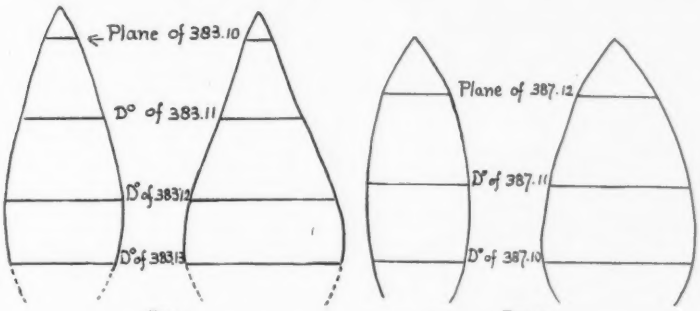

FIG. 1 FIG. 2

FIGS. 1, 2.—Showing slightly bifacial form of sporange as indicated by plotting series of transverse sections to scale on vertical line; fifth section in series (CN. 383. 14) just included five separated bases of loculi series, a fact on which the length (4 mm.) is deduced.

longitudinally through a single sporange, they are both somewhat peripheral, as the size of the body scarcely admits of two longitudinal sections. Both transverse series and longitudinal sections show that the sporange was almost always somewhat injured at the base where it has become detached from the parent plant.

ANATOMY.—The whole sporange is surrounded by a large-celled epidermis. The contents of the epidermal cells are carbonized, and from the mode of preservation of mucilaginous tissue in the Pettycur deposits, this blackened condition of the epidermis evidently precludes the view that it was mucilaginous (fig. 1). Beneath the epidermis is the hypoderm, composed of about five layers of cells. This tissue is continuous with the interstitial tissue between the loculi. In this cortical region are frequently found,

alternating with the peripheral loculi, especially near the apex and base, the characteristic vertical sclerotic plates of *Heterangium Grievii* (figs. 3, 4, 9, 13).

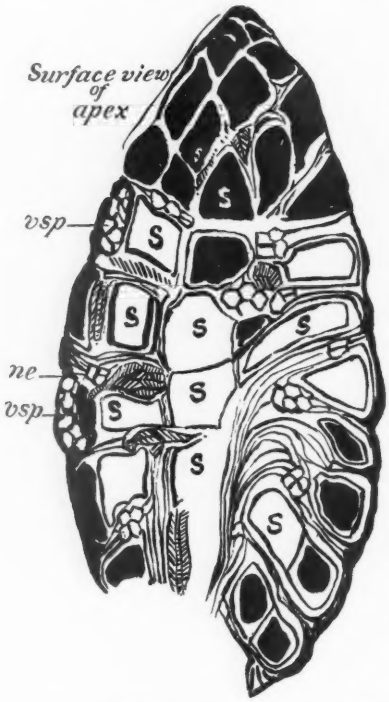

FIG. 3.—Diagrammatic drawing from two slightly oblique longitudinal sections (CN.386.17, 18), latter of which is nearly radial at base, and former superficial for upper third; it illustrates the somewhat spiral direction of locular series, preservation of spores in some but not in all loculi, vertical *Heterangium* sclerotic plates on left, large amount of water storage tissue and connecting tubules which abut on plates as in cortex of *Heterangium* petioles; sections were selected because of presence of ripe spores; central region is injured.

LOCULI.—The peripheral series of loculi which run longitudinally and somewhat spirally are more numerous than the loculi of *Telangium Scottii*, numbering in the middle zone of the sporange twelve (figs. 3, 9; cf. also text fig. 4). Their approximation into groups is sometimes seen near the apex (CN.383.10). Immersed in the ground tissue of the central region of the sporange, except at the extreme apex, there are further loculi, of which at least four appear in transverse sections (figs. 3, 12). Text fig. 3 is drawn from longitudinal sections too near the periphery to give the number and disposition of central loculi. The origin of additional central loculi by segmentation of peripheral loculi is suggested in some cases (text fig. 4), and the presence of ripe spores almost uniformly distributed in figs. 11 and 12 (CN.307.17, 18) suggests that the main content of the young normal sporange was potential sporogenous tissue. Each loculus is surrounded by a sheath of small, longitudinally ex-

tended, narrow cells. These sheaths, probably as a result of the shrinkage of the tissues due to carbonization, often become separated from the tissues which must have abutted on them in the living state. In the case of the central loculi the sheaths are often ruptured or entirely dissolved away, leaving the spores among the elements of the ground tissue (figs. 5, 11, 12). Small sclerotic plates often become incorporated with the walls of loculi, and when seen in profile are very characteristic (text figs. 4, 6, s^1p^1).

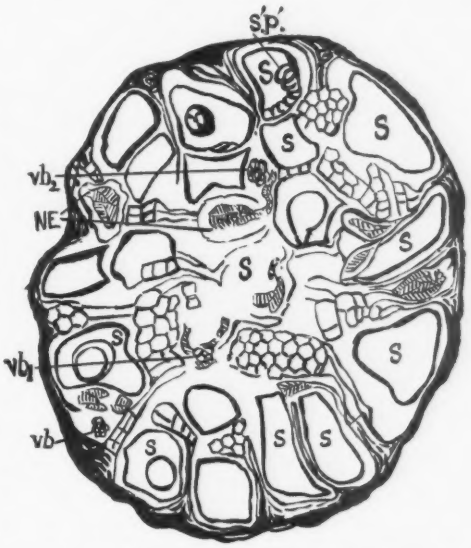

FIG. 4.—Diagrammatic drawing of transverse section of class II synangium (CN.411.31); swollen ground tissue not entered in detail; two deeply lying vascular bundles shown in transverse section (vb_1, vb_2); one peripheral vascular bundle also cut transversely on left, but most are cut so obliquely that they are merely indicated by hatching; nerve ending seen near center with its delicate branch strand; one or two central loculi are broken down, and carbonization of peripheral ones probably explains absence of spores in some (cf. fig. 5); at s^1p^1 a plate occurs attached to a loculus wall and is seen in profile as in CN.307.18, for which consult text fig. 6 and fig. 12.

In one synagium (figs. 11, 12) every loculus contains numerous ripe spores of tetrahedral form, similar in size and character to those in the pollen chamber of *Sphaerostoma ovale* (CN.270.1) among specimens of which it lies (text fig. 5). The spores measure 29 μ in their transverse and 20 μ in their radial extent. In this specimen some of the locular walls have broken down, but many of the peripheral and at least two of the central loculi are still intact. In other cases spores can be demonstrated only in some of the loculi, while others show opaque black or dark brown

contents, a condition which is possibly the result of the conditions to which the sporange was exposed during fossilization. As a rule the spores are better preserved in the interior than near the surface, which may be due to the conditions of exposure being slightly different. In a large number of cases the contents of the loculi have entirely been dissolved, or are in an abnormal condition. Such are the series CN.383.10–13 and CN.386.11–12 (figs. 1–4).

FIG. 5.—Camera drawings of spores from loculi in CN.386.18 and CN.307.17; tetrad in middle is characteristic and possibly indicates residuum of spore mother still persisted as in recent cycads; spores on left drawn from those in pollen chamber of *Sphaerostoma* for comparison (CN.270.1).

In the series CN.391.23–27, slide 391.26 shows in two cases a nerve ending in the center of the abortive loculus. In a large number of cases the peripheral loculi have been carbonized, but not the central ones. Swollen nerve endings accompanied by the *Heterangium* sclerotic plates appear to occupy the whole of the core of the sporange in the most advanced phyllodic specimens (text fig. 7). I refer to the partially or entirely abortive specimens as "phyllodic," as their tissues approximate the tissue elements of the ordinary sterile petiole of *Heterangium*. Moreover, they are linked together as in the petiole. There is a considerable range of form and size in the plates; some sporangia even seem to have produced more plates than others (fig. 12).

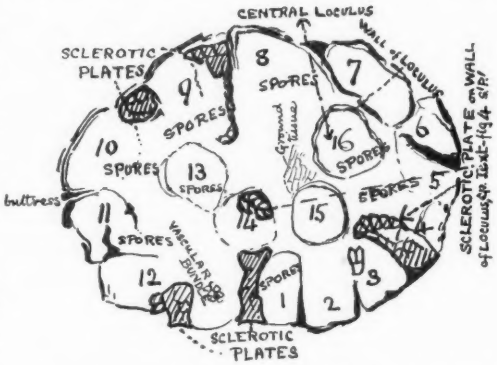

FIG. 6.—Key to fig. 12, showing outline of sclerotic plates, etc., which are clearer in slide than in micrograph.

The vertical plates in the periphery often have their cells thickened with layers of cell wall substance which almost obliterate the lumen of the cell; other plates are small, but most show the hexagonal form of cell indicated in text figs. 3 and 4. Abutting on these are tubular cells which are disposed mainly in the horizontal or transverse plane (text fig. 3). In the fossil these are generally swollen, and owing to their form and pale color have somewhat the appearance of macaroni. Some fibers accompany the delicate branches of the vascular bundles, and are continuous with a parenchymatous hood-like envelope to the nerve ending. Many, perhaps more in some specimens than in others, show a spiral line of thickening which resists imbibition and consequent swelling.

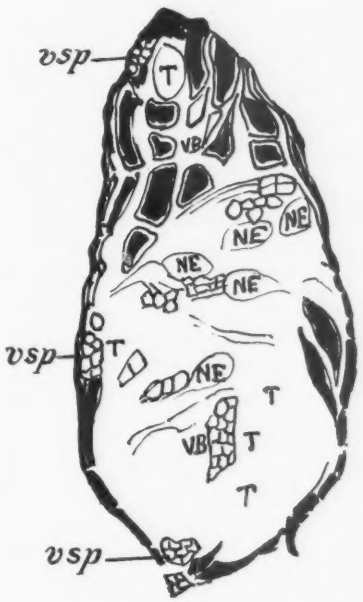

FIG. 7.—Camera sketch of specimen of *Heterotheca* in CN. 288. 2; no carbonaceous matter is retained in center, and therefore lignified elements of ground tissue appear exceptionally clearly; five nerve endings, many transfusion elements, and two vascular bundles can be distinguished in original; distribution of vertical plates (*vsp*) characteristic; striated elements seen near apex as in many cases (cf. text fig. 3).

The six to eight mesarch strands of primary xylem which travel mainly in the peripheral region give off the delicate centroscopically directed xylem strands, which end in the aqueous storage tissue. The contrast between the short barrel-shaped tracheids of the nerve ending and the slender carriers of the water is quite reminiscent of highly organized water storage organs in recent plants. The majority of the nerve endings in the synangium were directed to its central region. Transfusion elements of varying degrees of specialization are exceedingly abundant in some speci-

mens, and in some phyllodic specimens, such as that outlined in text fig. 7, where the carbonaceous matter is dissolved away in the center, the wide extent of the striated, possibly lignified, transfusion elements of the nerve endings is very clearly seen by the aid of the microscope. The nerve endings appear here to have wholly usurped the position of the central loculi (cf. fig. 6). The enlarged nerve ending is a familiar feature of the sterile pinna of *Lyginopteris*, and probably occurred in that of *Heterangium* also. The macerated condition of some of the specimens may be explained by the conditions to which they were subjected during fossilization. If we accept GORDON'S explanation (**8**) of the mode of formation of the Pettycur plant deposits, we may attribute it partially to the action of thermal springs and warm pools in which the accumulation of the plant remains were deposited and gradually infiltrated.

Text figs. 3 and 4, which illustrate the preceding statements, are both mere diagrams based on definite sections showing ripe spores. The positions in which the latter are clearly present have been indicated by *S*. They were probably present in other loculi, but were welded together into an impenetrable mass, owing probably to the presence of some vestige of the spore mother cell, which, as in recent cycads, appears to have remained long enveloping the tetrad. In the specimen shown in figs. 11 and 12 the spores are free, and there is no welding together of the contents of the loculus. In text figs. 3 and 4 sclerotic plates are indicated by groups of hexagonal cells. Two vertical plates can be seen in text fig. 3 (*sp*), and where plates are incorporated with the wall of a loculus they are indicated by s^1p^1. Larger horizontal plates are also seen in text fig. 4 in the central region. Vascular bundles, their delicate ramifications and large nerve endings, are indicated by hatching. The wall of a loculus has apparently broken down, as shown in the center of both text figs. 3 and 4, and spores are found among the swollen fibers. In both diagrams there is strong suggestion of segmentation of loculi, as Professor BOWER kindly pointed out to me.

Although the distribution of the vascular strands is mainly peripheral, at least two may be found in the foci of the ellipse formed by a cross-section (text fig. 4, vb_1, vb_2), but the number cannot be

regarded as determined. The peripheral strands probably alternated with every two series of loculi, but owing to the obliquity of their course (fig. 10 and text-fig. 7) they are often difficult to distinguish. An interesting point in the structure of the sporange in detail is the absence of any evidence of the building up of the synangium by a fusion of sporangia. The whole body strongly suggests its origin from a single sporange which has shared in the same type of skeletal elements as the petiole on which it was borne.

The sporogenous regions were distributed fairly equally throughout the body, except that their disposition in the periphery probably was determined by the approximately dictyoxylon type of cortex. If the loculi appear to be of unequal size, this is due partly to the varying girth of the sporange, and partly to the plane of section through the loculus. Wherever a section occurs in the surface plane, as in the upper third of text fig. 3, the buttresses are seen to lie so obliquely that a transverse section necessarily cuts through the contained loculi at different angles, and explains the fact that a transverse section does not differ much in appearance from a longitudinal one, except in form.

GROUPING OF SPECIMENS.—The available specimens, roughly sixty in number, may be grouped in four classes:

CLASS I.—Specimens such as that occurring in CN.307.16–19 (figs. 11 and 12), where the vegetative tissue is subordinate in amount to the sporogenous tissue. Such specimens show septa, mesarch vascular strands, and only a few water storage elements. The sclerotic plates characteristic of *Heterangium* in such sporangia are well developed, both in the periphery and in connection with the deeper lying septa.

CLASS II.—Specimens such as those occurring in CN.411.30, 31 and CN.386.17, 18 (figs. 5, 10). These contain ripe, apparently normal spores and normally disposed loculi, but undoubtedly a larger proportion of vegetative tissue than those in class I (text figs. 3, 4). The following series also belong to this class: CN.386.7, 8; CN.395.5, 6, 18, 19; CN.396.4, 5.

CLASS III.—Specimens such as those occurring in CN.383.10–13 and CN.386.11, 12 (figs. 1–4, 8, 9). Although one of these is possibly an immature form showing carbonization of the spo-

rogenous tissue within the loculi, for example, CN.386.11, 12, it is not possible to be sure of the nature of the carbonized contents.

No recognizable spores occur in any of the specimens included in class III, and in several series of sections which exhibit the structure of the body from apex to base, such as that in CN.391.23–27, the loculi seem to be filled up by concentric series of blackened cells, and in some cases have a well defined nerve ending in the center (CN.391.26).[2]

The majority of the available specimens belong to class III, which may be taken to include also specimens such as that in CN.393.6*A* (fig. 13), in which vegetative development obtains to such an extent that the conclusion is obvious that they were abortive or almost completely so. These specimens are of value as demonstrating the extreme condition of the phenomenon, and render probable the suggestion that there was some degree of phyllody (approximation to pinna structure) even in those of class II. The sections showing vegetative development are sometimes slightly larger than those in the normal series CN.307.17–19.

Significance of specimens in classes II and III

The explanation of the state of these sporangia, purely on the assumption of partial sterilization and maceration before impregnation with calcite, is not wholly satisfactory. In recent plants, when sporangia abort, the process affects the sporogenous tissue mainly, as in some specimens of *Angiopteris* sporangia, where the wall and apical crest (annulus) remain unaffected. A mucilage gland results in several familiar instances (**7, 16**). In the specimens under consideration, however, the loculi show no sign of a mucilaginous content, but, together with the septa, are represented by a considerable amount of tissue comparable with that in the vegetative pinnae of *Heterangium*, the parent plant.

KIDSTON and LANG (**10**) point out that the sporange in *Rhynia* and *Hornea* may be regarded as "corresponding to the tip of a branch of a thalloid sporophyte, inclosing sunken tetrasporangia."

[2] Tracheids possibly formed from degenerating sporogenous tissue of the ovule have been recorded by TREUB (**17**) in *Casuarina*, and by the writer (**2**) in *Castanea*, but it is an unusual phenomenon.

It is not an organ *sui generis*, but partakes of the same potentialities as other branches.

The widely accepted view that the Sphenopterid leaf is a meriphyte, and that the pinna corresponds with the cladodified primary branch axis, makes it easy to conceive of the microsporange showing in some cases the same anatomical elements as the pinna. The Sphenopterid type of frond as recorded by ARBER (2) had already made its appearance in the Devonian, and *Heterangium Grievii* may well have retained some reminiscence of the earlier unspecialized condition from which we conceive the sporange to be evolved. The curious specimens under discussion, which probably owe their preservation in a fossil form to their condition (as only two or three specimens of class I are so far recorded), are thus of considerable interest for the pinna-like character they exhibit. Nor is their more or less sterile condition without significance in any discussion as to the origin of the seed habit in *Heterangium*. Any comparable instability in the homosporous sporangia of the ancestor could well have been the beginning of a differentiation into megasporangia and microsporangia. A plant bearing vascular sporangia which retained but a few tetrads and relatively much surrounding vegetative tissue might well be regarded as on the high road to seed formation.

Evidence of synangial origin of seed

It must have occurred to many morphologists that a seed was a transformed synangium, the nucellus representing the one persisting fertile loculus, and the inner integument the vestigial peripheral part. In 1904, the evidence then available for this theory of the synangial origin of the seed was discussed by the writer (4) in connection with the description of *Telangium Scottii*, a synangium very common in the Coal Measures of the Upper Carboniferous rocks of the north of England, and the first species of that genus to be described showing structure. There were at that time certain difficulties in the general acceptance of the theory, chiefly because the structure of *Telangium* was such that, while explaining the integument, the absence of any central loculus rendered the explanation of the nucellar or central fertile loculus of the seed difficult.

Thus, as the absolute parallel between seed and synangium could not then be demonstrated, there were critics who preferred to lay stress on the possibility of the seed having acquired its characteristic structure independently of its ancestral condition, rather than by a transformation of structures already present. The inner integument, as well as the outer or indusial envelope, was attributed to foliar upgrowths around the nucellus, which alone was regarded as the representative of the ancestral sporange. The views of such critics are summed up by OLIVER (13) in his account of *Physostoma elegans* in the following words:

> The synangial theory, though no doubt tenable, presupposes in the ancestor the existence of the exceptional condition of a synangium, in which the peripheral members were ranged symmetrically around a central sporangium, and in which they persist as a sterilized envelope to form the seed coat.

Having at that time already secured several specimens which indicated the existence of a synangium with central loculi surrounded by peripheral ones, the writer took an early opportunity of collecting some more of the Pettycur deposits in which these interesting specimens occurred. By 1911, several hundred sections had been cut from two of the blocks collected the previous year. These blocks contained much *Heterangium Grievii* material, and yielded the large number of specimens now available of the new synangium. Owing to the care with which the stones were cut, many of the synangia, although scarcely 4 mm. in length and 2 mm. in width, are represented by series of three or four sections in the transverse plane or two in the longitudinal plane. The average thickness of the stone involved in the section cutting was 0.8 mm.

As already stated, the series of transverse sections CN.307.17–19, CN.343.10–13, and CN.412.30–31, although cut from different blocks, demonstrate clearly with many others that a synangium was present in these older rocks, associated with and showing the characteristic cortical structures of *Heterangium Grievii*, a synangium in which central loculi, as well as the more commonly occurring peripheral, occur. It is also interesting that even in the microsporangial apparatus an unusual amount of vegetative tissue obtained was also clearly shown for the first time. The publication of the details of the structure of *Sphaero-*

stoma in 1914 was to have been followed by the account of the microsporange *Heterotheca*, but other things have claimed attention, and it has only been since November 1918 that the work has again been taken up. The investigation of a larger number of specimens has thrown new light on the structure of *Heterotheca*, especially on the character and differentiation of the non-sporogenous tissue. With the possible exception of *Crossotheca* (text fig. 8, II), it differs from all other described microsporangia, and approaches what must be assumed to be a synthetic type of seed and microsporange. It is a succulent, sclerotic, vascular synangium, with central as well as peripheral loculi, and exhibits in its own tissues most of those found in the seed.

If we may have even in sporangia coexistent in the same species of plant a large amount of vegetative tissue, it is obvious that there is ground for assuming that in the early phases of evolution of the seed this would have been available as an envelope to the central fertile loculus, if the peripheral ceased to form sporogenous tissue.

A difficulty some have had in accepting the theory of the synangial origin of the seed has been expressed by the statement that "nothing useful has ever been produced by a sterilized sporange." It is granted that abortive sporogenous tissue generally eventuates only in a mucilage cavity, as COULTER and LAND (7) and STARR (16) have shown in particular cases, but useful structures certainly can be formed by the elaboration of the vegetative tissue surrounding masses of sporogenous tissue, as has recently been demonstrated (6) in the megasporange of *Mazocarpon*. It is not altogether sterilized potential sporogenous tissue of the peripheral loculi in *Heterotheca* which formed the inner integument of the ovule (canopy), but mainly an elaboration of the vegetative tissues which originally surrounded those masses of sporogenous tissue and finally supplanted them. For example, *Azolla* and all such leptosporangiate ferns were foredoomed to failure in the construction of a seed on these lines, as their peripheral sporangia had in all probability lost their vascular supply and their skeletal tissue before heterospory was evolved. The parallel to the seed habit which may be noted in *Azolla* is limited to the fact that the megasporange is one-spored, and occupies a central position in the sorus.

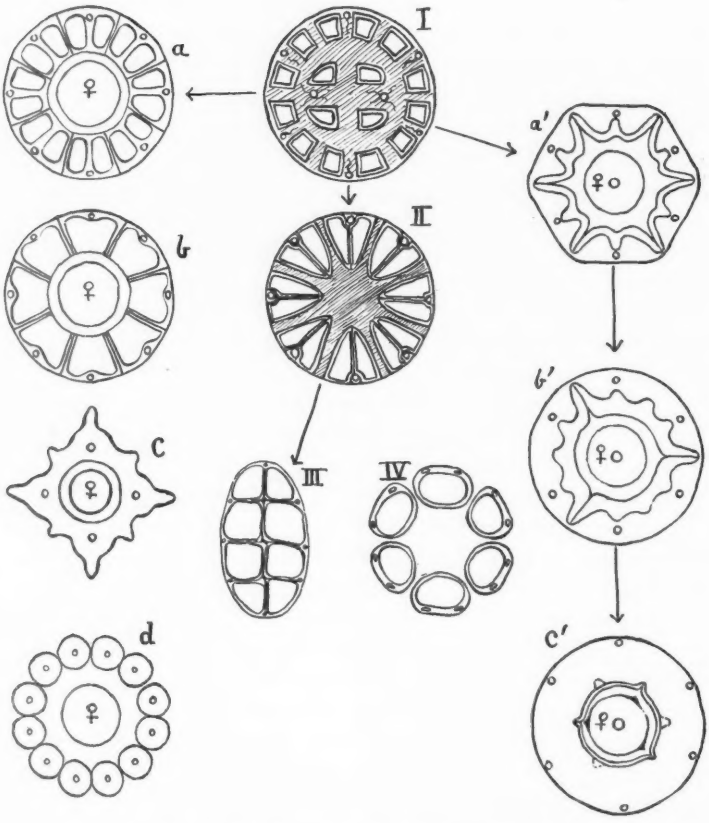

FIG. 8.—Diagrammatic transverse sections of various Pteridosperm ovules and pollen sacs to illustrate numerical relations they show to *Heterotheca* and one another: on left are *a*, *Sphaerostoma; b*, *Lagenostoma; c*, *Conostoma; d*, *Physostoma;* on right are a^1, *Polylophospermum;* b^1, *Trigonocarpus Parkinsoni;* c^1, *T. Shorensis;* in center are *I*, *Heterotheca Grievii; II*, *Crossotheca Hoeninghausi; III*, *Telangium Scottii; IV*, *Codonotheca caduca.* Vascular bundles indicated by small ring in each; arrows denote different phyletic lines; diagrams have been adapted from those of SCOTT and MASLEN, OLIVER, OLIVER and SALISBURY, SELLARDS, and three of writer's, references to which occur in text; relative size of bodies concerned has been neglected for convenience of comparison.

Lastly, a difficulty in the synangial theory of the seed has often occurred to the writer, but she has not heard it expressed by others. It is that in the compartments of the canopy of *Lagenostoma* a vascular bundle occupied a central position instead of, as one might have expected, a position in the plane of the lateral walls. As will be seen in text fig. 8, this difficulty is completely removed by the structure of *Heterotheca* and *Sphaerostoma*. Each compartment in the canopy of *Lagenostoma* is equivalent to a pair of loculi, which, although completely merged in *Lagenostoma*, can still be faintly traced in *Sphaerostoma*.

We owe much to Professor OLIVER for the open expression of his views published in 1909. The conclusions he finally arrived at were opposed to those which seem necessarily drawn from our present knowledge. Both theories as to the origin of the seed were mere hypotheses in 1908. Unfortunately, the view that the canopy of *Lagenostoma* was the product of a cupular or indusial upgrowth, led to the further hypothesis that the *Physostoma* segmented integument was a relatively primitive form, and even contributed to a suggestion that *Physostoma* was perhaps the most archaic type of seed known, a suggestion wholly contrary to what is known of its geological history. In the light of recent work, these latter hypotheses necessarily fall together, and it is to be regretted that a recent writer (14) should have referred to them as facts. OLIVER merely claimed that certain conditions should be proved to exist "in the ancestor," but evidently the necessary conditions have persisted in *Heterangium*, so that they coexist with the formation of an ovule for the megasporangial apparatus. Thus a link is provided between an ovule and a microsporangium which is stronger than was demanded, and there can be no longer any question as to the seed of a Pteridosperm, a seed that may well have been the homologue of all Pteridosperm seeds, having been produced in the course of evolution as a transformation product of a synangium. In this investigation we thus stand upon the threshold of the origin of at least one group of the Spermatophyta, and find more indications of it than were expected in the structure of a plant of the Lower Carboniferous.

The following is a more concise statement of the features of resemblance between *Heterotheca Grievii* and various ovules and microsynangia regarded as homologous with it. For the better elucidation of the subject, text fig. 8 has been constructed showing the possible numerical relations, etc., between the various sporangia of the Pteridosperms. We will commence with a comparison of *Sphaerostoma* and *Heterotheca*.

Heterotheca Grievii resembles *Sphaerostoma ovale* in the following particulars. It is approximately of the same form and dimensions (length 3.5–4 mm., width averaging about 2 mm.). It has probably six peripherally placed bundles in the buttresses between every two loculi as seen in transverse section. Besides these, there are two in the foci of the ellipse of the cross-sectional area, making eight in all, as in *Sphaerostoma*, although in the latter all eight are peripheral. The bundles are accompanied by enlarged water storage elements, and branch as in *Sphaerostoma* (5), fig. 3*a*. There is a central fertile region in each. In *Heterotheca* there is a large amount of vegetative tissue in the region between the loculi, forming buttresses vertical to the surface similar to those of the canopy of *Sphaerostoma*.

The differences can be explained by progressions of well known type. Thus the overarching of the central region by the peripheral at the apex to form the micropyle and sinus may have been partly in relation to the necessity of harboring the pollen grains, and partly a direct result of the freer vegetative development of the periphery. The later segmentation of synangia is a familiar phenomenon in Pteropsids, as may be seen by a comparison of *Angiopteris* and *Marattia*. The regular circumscissile dehiscence of the pollen chamber may be reminiscent of the time when there was a whorl of central loculi such as still exist in *Heterotheca*.

That there should be so few differences and so many resemblances between the microsporange and the ovule of the same species, makes it impossible to homologize merely the nucellus with the microsporange; as already stated, we must accept the synangial origin of the ovule as a whole, and regard the nucellus as derived from the central part of the common ancestor of both *Heterotheca* and *Sphaerostoma*. That the two structures, borne by the same species,

should be homologous is undoubtedly the simplest explanation of their origin. They both separate from the frond, and in this respect differ from later forms, where, on the attainment of dry dehiscence in the microsporange, the pollen escaped before the fall of the sporange. The indusium or cupule which surrounds the ovule as long as it remains on the parent plant is one of the distinctive features of microsporange and ovule, for there is no evidence of an indusium surrounding *Heterotheca*. This difference, however, is really confirmatory of the synangial origin of the inner integument, as such an origin does not involve a double indusial formation in a relatively primitive ovule. On grounds such as these, it may be assumed that in the particular case of *Heterangium Grievii* its microsporangia and ovules are homologous, both being transformation products of a common ancestor.

Those who accept this thesis will undoubtedly be prepared to accept it for all the other Lagenostomales, such as *Lagenostoma*, *Conostoma*, and *Physostoma*. A necessary corollary as respects *Physostoma* is that there has been a meristic variation in the periphery. The bundles are approximately, or we might say, potentially twice as numerous as in the periphery of such a sporange as *Heterotheca*, and each dominates one radially symmetrical "tentacle" instead of a dual compartment as in *Spharostoma* (text fig. 8, *a*, *d*). Thus instead of regarding *Physostoma* as a *Lagenostoma* with unfused chambers (OLIVER 13), we should, in the light of *Heterotheca*, regard it as a *Lagenostoma* with a lobed canopy.

Turning from the Lagenostomales to the Trigonocarpeae, which are regarded as the ovules of some at least of the Neuropterideae, we may ask if they bear any internal evidence of origin from such a type of synangium as *Heterotheca*. They show obvious series in the structure of the integument, series which in some respects run parallel to those in the Lagenostomales. Excellent details of the integument will be found in SALISBURY'S (14) work on *Trigonocarpus Shorensis*. In the Trigonocarpeae the nucellus is free from the inner integument except in the plane of its basal attachment, through which runs a vascular bundle which eventually forms a tracheal envelope round the nucellus. Six other bundles enter the integument and travel in its periphery. *Polylophosper-*

mum, *Trigonocarpus Parkinsonii*, and *T. Shorensis* show an obvious series in the reduction of the compartmental character of their integument (text fig. 8, a^1, b^1, c^1). In the two former the six bundles obviously alternate with compartments as in *Sphaerostoma* and *Heterotheca*, but in *T. Shorensis* all trace of the buttresses in the plane of the bundles has disappeared, as in *Lagenostoma*, and the bundles appear to occupy a central position.

The numerical relations of the bundles in *Heterotheca* and the Trigonocarpeae correspond, if we may assume in the latter a simple fusion of the two central bundles to supply the megasporange (nucellus), which in this series of seeds is free from the integument, and thus necessarily requires a central water supply. The tracheal mantle which becomes such a prominent organ in some of the Neuropterid seeds, for example, *Stephanospermum* (OLIVER **12**), needs no explanation if the nucellus were derived from an ancestral sporange such as *Heterotheca*, with its large water storage equipment. Further, the succulent sarcotesta of the Trigonocarpeae is extraordinarily similar in structure to the succulent ground tissue of *Heterotheca*, so far as respects the macaroni-like tubules (text fig. 3). There is thus a considerable sum of evidence in support of the homology of the Pteridosperm seeds with a microsynangium such as *Heterotheca*.

Let us now turn to a consideration of the other microsporangia attributed with general assent to Pteridosperms. I shall only refer to *Crossotheca Hoeninghausi* (KIDSTON **9**), *Telangium Scottii* (BENSON **4**), and *Codonotheca caduca* (SELLARDS **15**). Of these, *Telangium Scottii* is the only one in which the details of the anatomy have been published, but the preservation of the other sporangia as incrustation fossils is exceptionally good, and it has been possible to learn much of their organization. *Crossotheca Hoeninghausi* shows eight peripheral pairs of loculi, each pair showing a single vascular bundle running up in its dividing wall. In the young condition the eight pairs are seen to be in close lateral approximation, so that they form a body resembling a stout undehisced *Telangium* with a wide base. In specimens recently provided by Mr. HEMINGWAY, there seems no reason to doubt that this younger condition (occurring nearer the tips of the fronds) represents an

undehisced condition of a synangium. In KIDSTON'S original description (9) the synangium was regarded as built up of eight discrete dual sporangia. In a transverse section kindly lent by Dr. KIDSTON, it was noted that the matrix within the ring of paired loculi was stained yellow, and this is suggestive of decayed ground tissue having been present originally where now only a boss of rock can be seen.

ZEILLER thought that the members of the whorl were sometimes coherent. If this be the true interpretation, *Crossotheca Hoeninghausi* occupies a position almost halfway between *Heterotheca* and *Telangium*. It resembles *Telangium* in the possession of a single peripheral series of loculi, in apical dehiscence, and its retention on the frond until the spores are shed. It resembles *Heterotheca* in the loculi being distributed one on either side of longitudinal discrete vascular bundles, in the possession of much sterile ground tissue, and in the number (sixteen) of its longitudinal loculi or loculi series. It differs from *Telangium Scottii* in the number of its loculi, and from *Heterotheca* in the absence of central loculi and in its apical dehiscence. I have no evidence as to the character of its ground tissue, but it is probable that it was non-cuticularized.

From these considerations I have included in text fig. 8, II an adaptation of KIDSTON'S fig. 9, indicating by hatching the supposed ground tissue which had perished in the incrustation. It would seem best to retain the name *Crossotheca*, as the body is sufficiently distinct from any species of *Telangium* to retain its separate form genus rank.

Codonotheca caduca is regarded by SELLARDS as probably a Neuropterid sporange. It is fairly safe to regard it as belonging to some Pteridosperm, and a reference to it may be useful. Like the *Trigonocarpus* seeds, it is provided with six peripheral bundles, but in this case each becomes duplicated. Like the canopy of *Physostoma*, the peripheral series have become lobed. There are no central loculi, which are so far only recorded for *Heterotheca*. *Telangium Scottii* has but half the number of loculi recorded for *Crossotheca*, a mutation probably having occurred comparable with that which gave rise to the reduced number of bundles of *Conostoma*, which shows but four (text fig. 8, *c*). The three series

shown in this figure, for example, the Lagenostomales (*a–d*), the Trigonocarpeae (a^1–c^1), and the microsporangia (I, II, III, IV) illustrate the fact that at least two lines of progression can be traced in Pteridosperm seeds, each with members suggesting an early descent from a sporange with paired loculi. These paired loculi occur in both *Heterotheca* and *Crossotheca*. A tendency to simplification is seen in all three groups, *Conostoma* among the Lagenostomales and *Telangium* among the microsporangia showing a halving of the number of parts. Again an example of lobing occurs in one seed of the Lagenostomales (*Physostoma*, text fig. 8, *d*), and in one type of microsporangium (*Codonotheca*, text fig. 8, IV).

Summary and conclusions

1. Among the remains of *Heterangium Grievii* (vegetative organs and seed) in the calcified deposits of plants occurring at Pettycur, Fifeshire, have been found large numbers of a new type of microsporange for which the form genus *Heterotheca* has been constructed.

2. Its mechanical structure is similar to that of the petioles of *Heterangium*, and, although so far found detached, it is attributed to *Heterangium* on the same grounds as are the vegetative organs, that is, the presence of vertical and horizontal sclerotic plates.

3. Its spore bearing tissue is distributed in sixteen series of loculi, of which twelve are peripheral and four central. The vascular bundles are similar to those of the seed, and near the apex each is nearly surrounded by a pair of loculi as in the canopy of the seed.

4. The structure throughout is strongly confirmatory of the homology of the seed and synangium, and is regarded as supplying ample proof of the synangial origin of the seed.

5. In this investigation we seem to stand upon the threshold of the origin of at least one group of the Spermatophyta, and the conclusions reached cannot fail to have their influence on the study of other groups.

ROYAL HOLLOWAY COLLEGE
ENGLEFIELD GREEN
SURREY, ENGLAND

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EXPLANATION OF PLATES IV AND V

The following abbreviations are used in the illustrations: *b*, buttress or interstitial tissue between peripheral loculi; *e*, epidermis; *g*, gap in stone section; *gt*, ground tissue; *l*, loculus; *cl*, central loculus, *ne*, nerve ending; *s*, spores; *sp*, sclerotic plate; s^1p^1, plate attached to loculus wall; *vsp*, vertical sclerotic plate; *vb*, vascular bundle.

PLATE IV

FIGS. 1, 2.—Two successive cross-sections of immature synangium; epidermis is preserved, and in center of body occur small sclerotic plates; no central loculi can be distinguished, and content of peripherous ones is amorphous; matrix has been left around fig. 1 (CN.386.11, 12); ×33 diameters.

FIGS. 3, 4.—Two successive cross-sections of synangium nearer base; central loculi well shown (CN.383.12, 13); ×29 diameters.

FIG. 5.—Transverse section of mature specimen, showing many of loculi with ripe spores, some of which, owing probably to solution of loculus wall, lie free in ground tissue; rough diagram of this section given in text fig. 4 (CN.411.31); ×37 diameters.

FIG. 6.—High power photograph of central ground tissue of obliquely longitudinal section, showing highly organized nerve ending (*ne*), various horizontal sclerotic plates, and succulent ground tissue like that of sarcotesta of Trigonocarpeae; in original, striated elements of transfusion tissue can be seen above nerve ending (CN.304.2); ×100 diameters.

FIG. 7.—Transverse section through upper part of synangium, showing central loculi and position of nerve ending (CN.411.12); ×18 diameters.

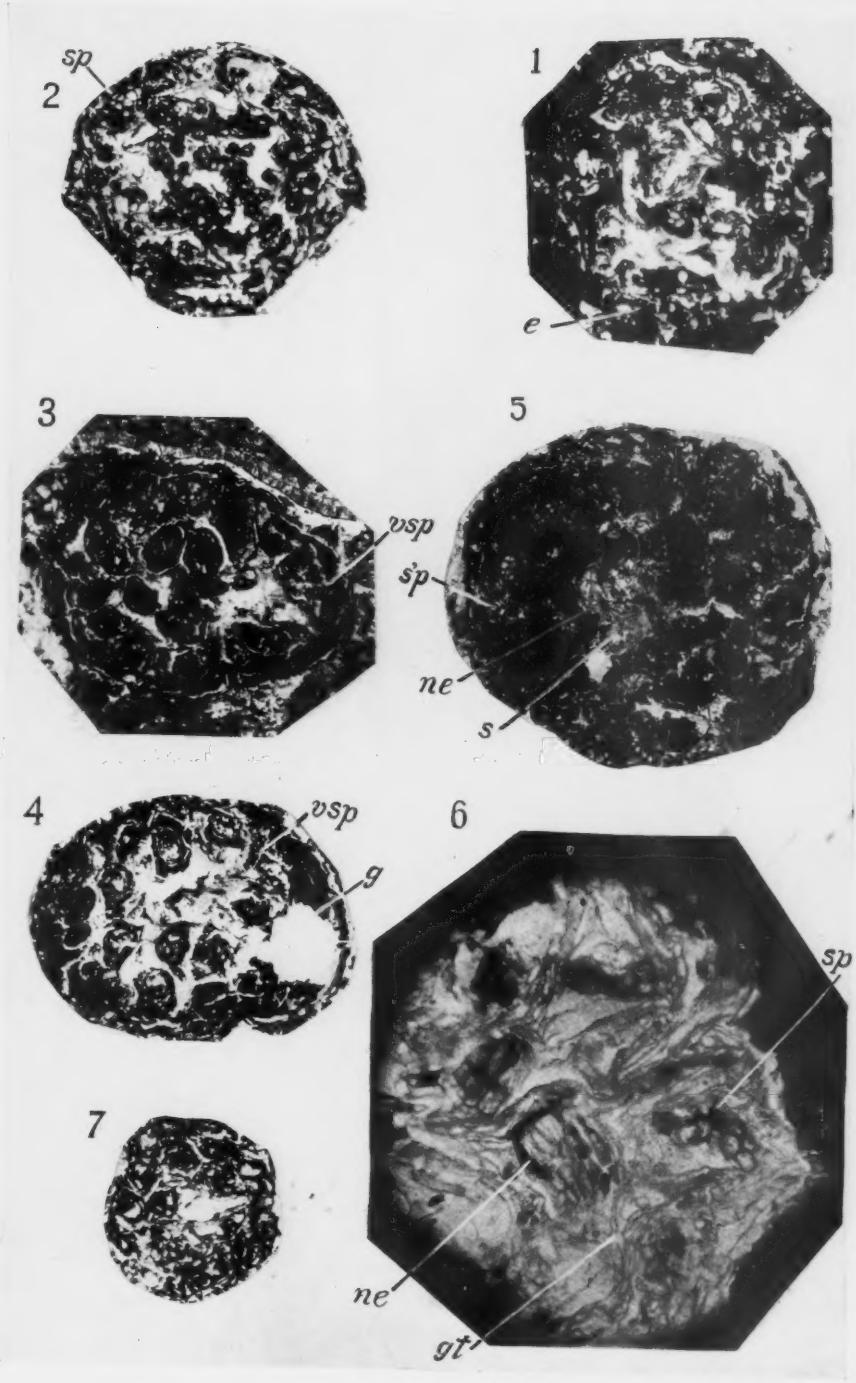
PLATE V

FIGS. 8, 9.—Two sections across another synangium, showing position of loculi in spite of some degree of maceration and subsequent shrinkage; in fig. 8 plane is nearer apex; surface is seen abutting on other tissues in matrix; in none of the four sections of this series are spores to be detected; fig. 8 (CN.391.23), ×25 diameters; fig. 9 (CN.391.24), ×40 diameters.

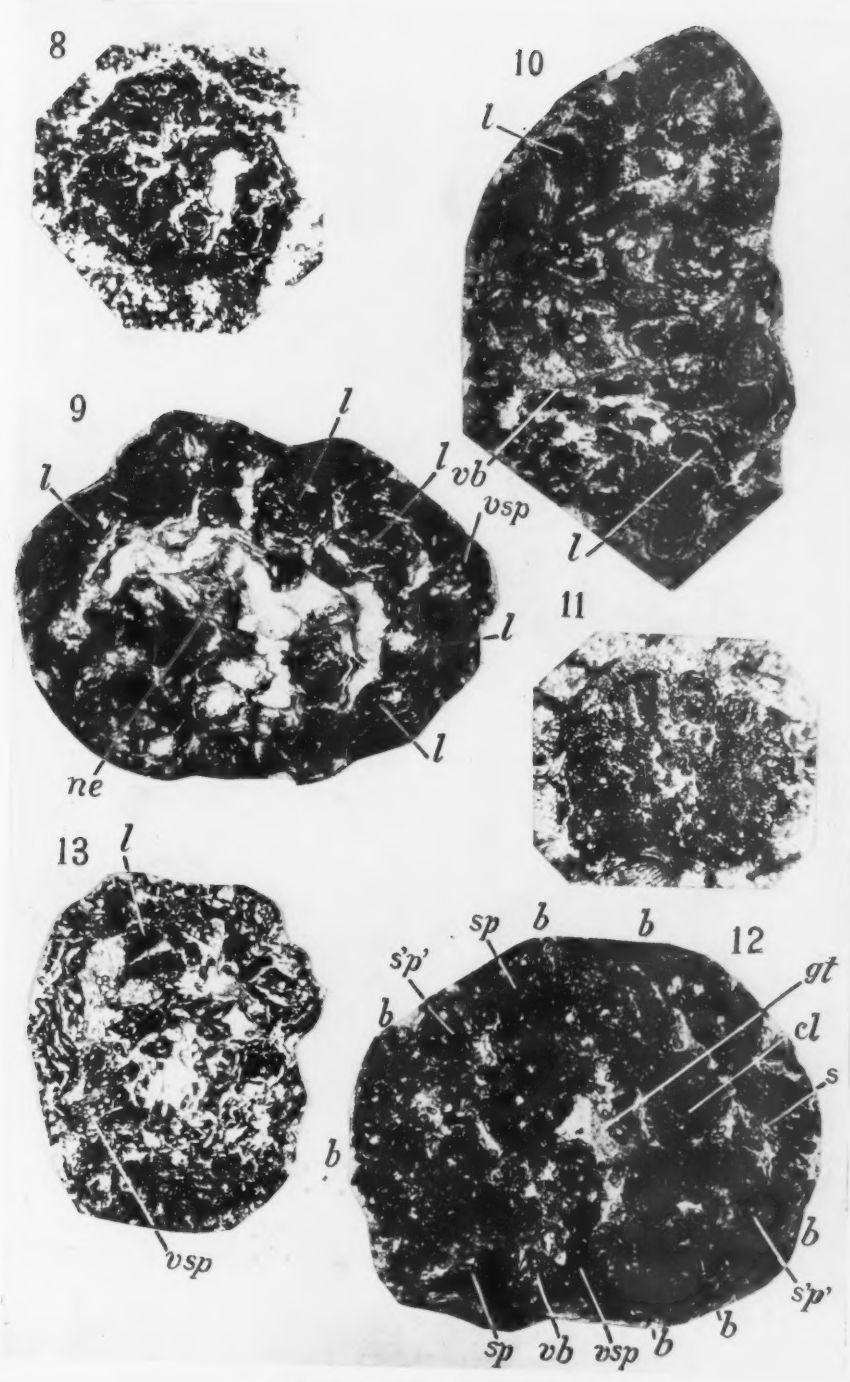
FIG. 10.—Nearly peripheral tangential section, showing in original the transversely oriented series of loculi, of which majority show tetrads of spores; cf. text fig. 3, constructed from two successive longitudinal sections; it is given to show transversely running strand (*vb*), for which reason the rest is slightly over exposed (CN.386.17); ×42 diameters.

FIGS. 11, 12.—Two successive transverse sections of class I synangium, in which spores are free from one another and all loculi are full of them; synangium had undergone some degree of maceration, as shown by sclerotic plates (*sp*), cells of which show partial solution of middle lamella; key provided for fig. 12 in text fig. 6, to indicate position of sclerotic plates, loculi, etc., which are clearer in the fossil, although many of the walls have given way; fig. 11 (CN.307.17), ×27 diameters; fig. 12 (CN.307.18), ×50 diameters.

FIG. 13.—Transverse section clearly showing vertical sclerotic plates (*vsp*); most of loculi have perished, but one is indicated (*l*); several others are easily detected in the slide (CN.393.6*A*); ×32 diameters.



BENSON on HETEROTHECA



BENSON on HETEROTHECA

EARLY EMBRYOGENY OF REBOULIA HEMISPHAERICA

(WITH FORTY-SEVEN FIGURES)

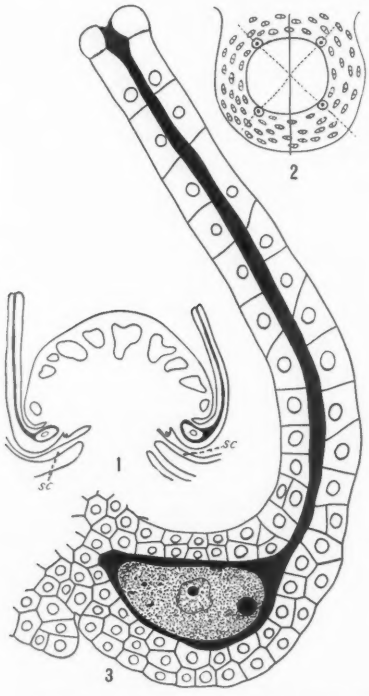
A. W. DUPLER

Our knowledge of the development of the sporophyte of *Reboulia* dates back to HOFMEISTER (11), who described its early stages and its rapid growth as it approaches maturity. KIENITZ-GERLOFF (13), who studied the embryogeny of a number of forms, claimed the *Reboulia* embryo to be similar to that of *Grimaldia*, certain stages of which he described in some detail, but not including the earliest stages. LEITGEB'S (14) work on the Marchantiaceae included a study of *Reboulia*, of which he described the development of the sporophyte in a general way. CAVERS' (3) observations also included the early and late sporophyte of *Reboulia*. The more recent work on the embryo has been done by WOODBURN (18) and HAUPT (10), the former dealing with the very early stages, the latter describing the development from beginning to maturity. These two recent accounts differ somewhat from the earlier studies, and in certain features from one another. A study of the writer's collections of material has yielded certain results which may be of interest in comparison with these accounts, especially where they bear on their differences, and in the addition of certain facts not mentioned by them. Altogether it is clear that the embryo of *Reboulia* shows considerable variation in the early phases of its development.

Material

The material for this study was collected near Huntingdon, Pennsylvania, the greater bulk of the embryos figured having been secured from collections made from the early part of October to the latter part of November, 1919. None of the material collected in September shows embryos so far as it has been examined. The early winter condition (figs. 46, 47) was secured from a collection made December 23, 1920. The material was killed in 25 per cent chrome-acetic acid, and for the most part stained by the iron-alum

haemotoxylin method. As DURAND (7) found in *Marchantia*, the embryo stains much lighter than the surrounding calyptra, and it was found that dipping the slides for a short time in an alcoholic solution of Lichtgrün served to bring out clearly the delicate cell walls. This clouds the cytoplasm of the cells to a slight extent, and is not to be recommended when the details of cell structure are to be studied.



FIGS. 1–3.—Fig. 1, vertical section of young female receptacle with mature archegonia; fig. 2, diagram showing relation of archegonia to receptacle and longitudinal axis of thallus (solid line); dotted line indicates median plane through bilateral archegonium and embryo; fig. 3, median longitudinal section of mature archegonium; dark area about embryo represents space between embryo and calyptra; fig. 3, ×400.

Archegonium

The writer has found nothing in the development of the archegonium differing from the account given by HAUPT (9). It first appears when the female receptacle is yet quite small, and when the sex organ is mature the receptacle is still a low conical structure (fig. 1), surrounded by a large number of sterile scales (fig. 2). The venter of the archegonium is inclined somewhat below the horizontal, the neck curving upward more or less to a perpendicular position among the scales. HAUPT (9) regards these scales as probably protective in function. They also probably serve in holding a film of water about the archegonia, functioning much as do the paraphyses about the sex organs of mosses, resulting in conditions which increase the probabilities of fertilization.

Usually there are four archegonia on each receptacle, one close behind the apical cell of each of the four growing points of the receptacle. Rarely three, occasionally five or six such growing points and archegonia may occur. In the typical condition the archegonia are so situated on the receptacle that a median section through the entire archegonium can be secured only by vertical sections cut on a plane at an angle of 45° to the long axis of the thallus (fig. 2). Owing to their curvature, both the archegonium and the early embryo are bilateral and not radial, and a strictly median section can pass through but one plane. With but few exceptions all the embryos figured in this account are from sections along this plane.

The egg at maturity is about twice as long as its transverse diameter, bluntly rounded at both ends, slightly more tapering at the hypobasal end, and with its long axis describing the arc of a circle (fig. 3). The nucleus is centrally placed, the egg cytoplasm uniformly distributed, and containing plastids and oil globules. There is usually a very conspicuous oil globule near the anterior end, which persists even in late stages of the embryo.

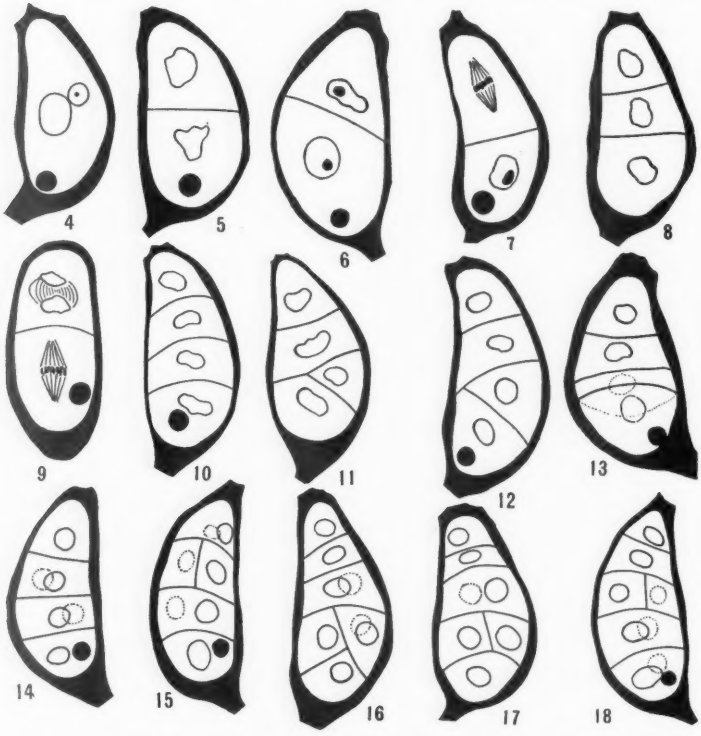
Fertilization

The close proximity of the male and female receptacles on the same branch of the thallus usually insures fertilization, although occasionally it fails to occur. WOODBURN calls attention to the change which the motile sperm undergoes from the time it leaves the antheridium until its nucleus is ready to fuse with the egg nucleus. The writer found a number of cases in which the sperm nucleus had penetrated the egg cytoplasm and lay close to the egg nucleus (fig. 4). At this time the egg nucleus has about twice the diameter of the sperm nucleus, whose more compacted chromatin results in a denser staining body. No attempt was made to study the nuclear changes involved in fertilization. Apparently the fusion nucleus passes into the resting stage before division of the egg takes place.

Embryo

FIRST DIVISION.—Without any considerable enlargement after fertilization, the egg divides by a transverse wall, usually perpendicular to the long axis, and giving nearly equal epibasal and

hypobasal cells (fig. 5). This agrees with the accounts for *Reboulia* as given by CAVERS (3), WOODBURN (18), and HAUPT (10), and with



FIGS. 4–18.—Fig. 4, egg with male and female nuclei, black circle representing oil drop; fig. 5, first wall transverse; fig. 6, first wall oblique; fig. 7, mitosis in hypobasal cell; fig. 8, embryo of three cells; fig. 9, mitosis in both epibasal and hypobasal cells; fig. 10, typical filamentous embryo of four cells; figs. 11–13, epibasal cell divided by oblique wall; fig. 14, vertical division of two middle cells of row, basal and apical cells undivided as yet; fig. 15, apical cell not yet divided by vertical wall; fig. 16, epibasal cell divided by oblique wall, vertical division in middle of embryo, basal cell of row of four probably transversely divided; figs. 17, 18, two cells at base probably resulting from transverse division of basal cell; ×430.

such forms as *Targionia* (CAMPBELL 1, O'KEEFE 16), *Plagiochasma* (STARR 17), *Conocephalum* (CAVERS 2), *Riccia* at times (GARBER 8), and practically all the Jungermanniales which have been examined,

among which are *Sphaerocarpus* and *Geothallus* (CAMPBELL 1), *Aneura* (LEITGEB 14, CLAPP 5), *Fossombronia* (HUMPHREY 12), *Pellia* (KIENITZ-GERLOFF 13), and *Symphyogyna* (MCCORMICK 15), and is in contrast with forms in which the first wall is more or less oblique, as occurs in *Riccia* (KIENITZ-GERLOFF 13, CAMPBELL 1, GARBER 8), *Marchantia* (DURAND 7), and *Preissia* (KIENITZ-GERLOFF 13).

HOFMEISTER thought the *Reboulia* egg divided first by a strongly inclined wall. LEITGEB claimed the first wall to be generally oblique, occasionally perpendicular, to the long axis. HAUPT (10) states that the first division is "always accompanied by a transverse wall." WOODBURN's statement is not so positive, and one of his figures shows the first wall slightly inclined. The writer found several cases in which the first wall was more or less oblique, sometimes with the epibasal cell the larger of the two (fig. 6).

FILAMENTOUS EMBRYO.—The published accounts differ considerably as to the behavior following the first division. HOFMEISTER described the growth as due to an apical cell with two cutting faces, the epibasal cell dividing repeatedly by alternately inclined walls, resulting in a slender embryo of two rows of cells. KIENITZ-GERLOFF was not convinced by HOFMEISTER's account, but concluded from analogy with *Grimaldia* that in *Reboulia* an octant is formed by vertical walls perpendicular to the first transverse wall. LEITGEB claimed quadrant formation by walls perpendicular to the first, the apical and basal cells of the quadrant being the larger, since the first wall is usually oblique, and both the epibasal and hypobasal cells are divided unequally. CAVERS (3) also claimed an octant by the formation of perpendicular walls, and regarded the epibasal half of the octant as giving rise to the capsule, the hypobasal to the foot and stalk. The studies by WOODBURN, HAUPT, and the writer do not agree with these earlier statements. In most cases the second and third divisions are parallel to the first, resulting in a filament of four cells, with the walls between them more or less parallel to one another.

WOODBURN and HAUPT both claim the second division to be in the epibasal cell, resulting in a row of three cells. WOODBURN says the third division may be in either the apical or the middle of these

three cells; HAUPT states that it is in the apical cell. According to both workers the first division of the hypobasal cell does not take place until after the formation of the row of four cells, and then usually by a vertical wall. HAUPT says that occasionally this vertical wall formation takes place before the third transverse division, that is, when the embryo consists of but three cells. Neither WOODBURN nor HAUPT show mitotic figures definitely proving this sequence of division. The sequence of these early divisions may hold a definite relation to the later differentiation of the sporophyte into foot, stalk, and capsule regions. The writer's preparations show that transverse division may take place in both hypobasal and epibasal cells (fig. 9), and that the division of the hypobasal cell precedes that of the epibasal cell (fig. 7). The division of the hypobasal cell may be completed before the epibasal cell begins to divide, resulting in a three-celled filament (fig. 8). Such embryos are probably quite rare, however, the writer having found but a single case. It is more probable that the division of the epibasal cell is generally initiated before that of the hypobasal cell is complete (fig. 9), and the four-celled embryo results with the completion of the two division processes (fig. 10). The writer concludes, therefore, that at this stage the embryo consists typically of a row of four cells with parallel walls, as a result of the transverse division of both hypobasal and epibasal cells, the division of the former preceding slightly that of the latter. Not a single case was found suggestive of the quadrant, as claimed by CAVERS, who probably based his interpretation on later stages without having observed these early ones, as he shows no figures of early embryo development.

Variations from the typical situation are of interest. For example, the transverse walls are often more or less curved, with the concave side toward the apex, the curvature often being especially pronounced in case of the apical cell (figs. 10, 17); or the division of the epibasal cell may be by an oblique wall (figs. 11–13) whose inclination to the perpendicular may show considerable variation. Such a division of the epibasal cell is more likely to occur when the first division has been an oblique one (figs. 11, 12), although the inclination of this wall may be independent of the first wall (figs. 13, 16). WOODBURN figures several embryos showing oblique walls

in the epibasal portion. HAUPT thinks oblique walls do not occur at this stage or later. It seems to the writer that the evidence for the occasional occurrence of oblique walls is conclusive.

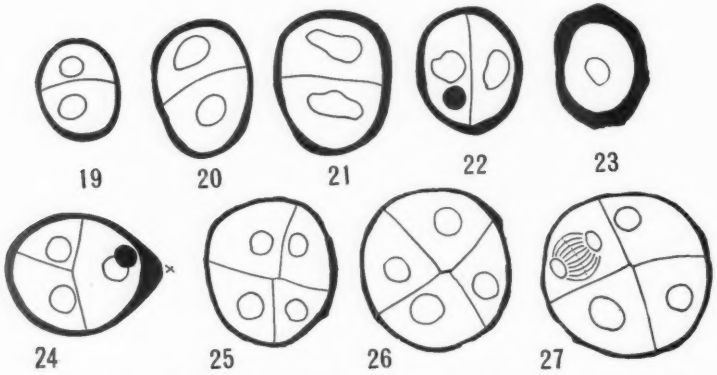
The occurrence of a filamentous embryo in *Reboulia* agrees with the embryo described for *Plagiochasma* (STARR 17), *Targionia* (O'KEEFE 16), *Geothallus*, and *Sphaerocarpus* (CAMPBELL), as well as practically all the Jungermanniales. Quadrant formation by walls vertical to the first wall occurs in *Riccia* (CAMPBELL 1), in *Marchantia*, as given by a number of writers, DURAND'S account being the most complete, *Conocephalum* (CAVERS 2), and *Fimbriaria californica* (CAMPBELL). KIENITZ-GERLOFF made a similar claim for *Grimaldia* and *Preissia*, but did not have the early stages. GARBER found occasionally a row of three cells in *Riccia natans*, although the quadrant form was the rule. In the following account the innermost cell of the filament of four cells will be designated as the basal cell, the outermost as the apical cell.

VERTICAL WALL FORMATION.—Vertical walls now begin to form in the young embryo. According to HAUPT, "these vertical divisions begin at the lower end of the embryo, a feature which is also noted by WOODBURN'S figures." This probably is the general rule, and is evidenced by HAUPT'S figures, which show mitoses in the hypobasal portion before occurring in the epibasal portion, the basal cell evidently dividing first. It is quite common to find embryos of this stage with the apical cell yet undivided (figs. 14, 15). This cell also soon divides, either by a vertical wall or otherwise, as described later. A series of cross-sections of an embryo at this stage shows that the vertical walls do not usually lie in the same plane, but are inclined to one another at various angles (figs. 19–23). These vertical walls are usually perpendicular to the transverse ones, which, if obliquely inclined, usually result in oblique vertical walls, and the embryo may, in surface view of this and later stages, appear spiral. Occasionally some of the vertical walls are oblique to the transverse wall, even in the middle of the filament (figs. 17, 28).

The first vertical walls are soon followed by a second series, usually at right angles to the first, typically dividing each segment into four cells (figs. 25, 26). These divisions may be more or less simultaneous (fig. 36), although not ordinarily so even in the same segment.

Should the apical cell also divide by the two series of vertical walls, the apex of the embryo will consist of four octohedral cells (fig. 33). This is the situation as described by HAUPT. LEITGEB describes a similar condition in *Blasia*. Owing to the curvature of the embryo two of these will be nearer the neck of the archegonium than the other two.

APICAL CELL.—The apical cell of the row of four may divide vertically, or, as certain embryos suggest, it may divide again transversely (fig. 29) before vertical division takes place, although in

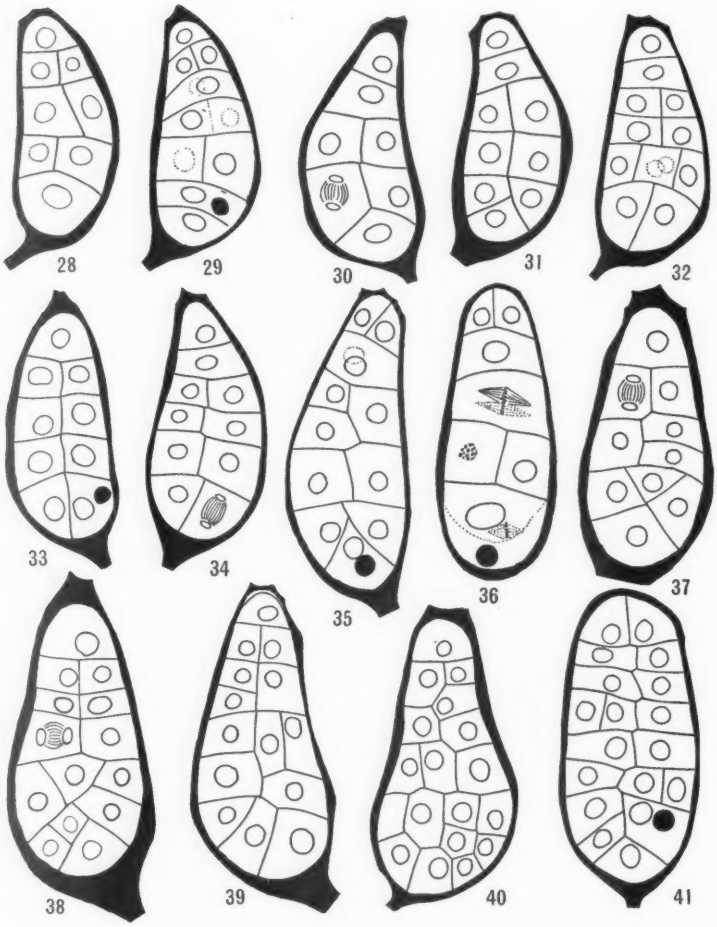


FIGS. 19–27.—Figs. 19–23, series of cross-sections through embryo, showing inclination of vertical walls to one another in successive segments; fig. 19 is basal segment; fig. 23, apex; fig. 24, transverse section of apical segment of embryo, *x* is neck of archegonium; figs. 25–27, transverse section of embryo with segments in quadrants; compare fig. 27 with fig. 42, noting position of walls; ×570.

the absence of a mitosis one cannot be absolutely positive on this point. It may also divide by an oblique wall, whether its basal wall is oblique or transverse (figs. 31, 32, 34, 35). WOODBURN shows a similar situation, to which HAUPT takes exception, claiming that in his investigation "a truly median section has never revealed the presence of a triangular apical cell." LEITGEB found that in *Blasia* the apical cell may divide by oblique walls, and he figures several embryos with a triangular apical cell. The writer's observations of *Reboulia* confirm WOODBURN'S statement. In fact, owing to the curvature of the embryo, a truly median section is the one

most likely to show the oblique inclination of the wall and the consequent triangular apical cell, although one would not necessarily err even in the interpretation of an oblique section. It seems to the writer that there is no room for doubt as to the presence and functioning of a triangular apical cell (figs. 34–39, 41, 45). It is not probable that this cell functions as an apical cell more than a very few times, being soon "lost" in the growing embryo, where the apical function becomes distributed to a number of cells (figs. 40, 46).

BASAL CELL.—The basal cell of the row of four also does not perform uniformly in all cases. It may divide by a vertical wall into two approximately equal cells (fig. 36), or it may divide by an oblique wall (fig. 35). WOODBURN found "basal cells of triangular shape" to occur, probably arising as a result of oblique wall formation in the basal cell. HAUPT found no case of this, and in the writer's preparations it is not common. Should the first wall of the divided egg be an oblique one, it might be probable that the hypobasal would divide obliquely, resulting in a triangular cell at the base. While no mitosis was found as a direct proof, the appearance of a number of embryos (figs. 16–18, 30–32, 34) suggests that the basal cell may divide transversely instead of vertically, the basal of the two cells thus formed behaving as here described for the basal cell itself, while the other cell sooner or later becomes divided by vertical walls in the same way as its neighboring segment (figs. 28, 29). Vertical wall formation in this cell would probably be delayed for a time and the cell remain undivided, even after vertical walls have formed in the segments anterior to it (figs. 34, 36). It seems to the writer that very frequently the basal cell of the row of four undergoes no further division whatever, but very early becomes differentiated as a large conspicuous foot cell at the base of the embryo, retaining its hemispherical shape, very early showing denser contents than the other cells of the embryo, and becoming coated on its free margin by a heavy thickening (figs. 45–47). This cell often remains quite distinct, even in late embryos, and may clearly be recognized both in sections (fig. 47) and in surface views of dissected embryos. Should the basal cell have divided transversely (as already suggested) the basal of the two cells formed may remain undivided. WOODBURN



FIGS. 28–41.—Fig. 28, embryo with segment divided by oblique wall; fig. 29, apical cell (of row of four), probably divided transversely; figs. 30–41, embryos showing varying features, figs. 36, 37, and 41 cut at plane perpendicular to median longitudinal plane; in fig. 36 division is going on at several different regions of embryo; dotted line at apex represents oblique wall separating deeper cell (in process of division) from superficial cell with oil drop and undivided nucleus; figs. 34, 35, 37–39 show functional triangular apical cell; ×430.

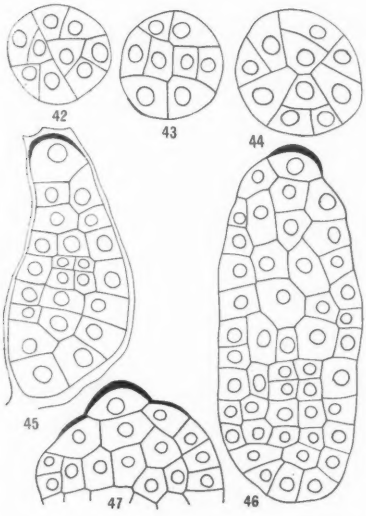
cites a case where the basal cell has divided into a small group of irregular cells, probably also a very rare feature.

It is evident that the basal cell of the filamentous embryo undergoes but few, and in some cases no further divisions, and therefore makes a relatively small contribution to the tissue of the sporophyte, which therefore is built up almost entirely from the three anterior cells of the filament of four cells.

LATER GROWTH AND EMBRYO DIFFERENTIATION.—Along with or following the formation of vertical walls, transverse divisions in some or all of the segments result in additional tiers of cells, the divisions occurring in different planes without any definite sequence (figs. 36–41). At first the ventral side of the embryo will probably show the greater number of cells (figs. 35, 39, 40), but as growth continues the dorsal side also grows rapidly, and the embryo soon becomes a radial instead of a bilateral structure (fig. 46). Periclinal walls now form, especially in the epibasal portion of the embryo, without any definite sequence, forming inner and outer cells (figs. 38–44). These first periclinal walls are most likely to form in the capsule-forming region of the embryo, which becomes considerably broader than the more slender hypobasal portion (figs. 40–45). This portion, however, soon broadens out somewhat and reaches the winter condition (fig. 46). While it is impossible to trace back absolutely the origin of the different regions of the sporophyte, it seems most probable from the writer's study that the first division of the egg determines the capsule region as distinguished from the foot and stalk region, the epibasal cell giving rise to the capsule, therefore, the hypobasal to the stalk and foot, with the bulk of both foot and stalk derived from the anterior half or three-fourths of the original hypobasal cell. They are derived from the anterior half if the basal cell (of the row of four) does not undergo transverse division, and from the anterior three-fourths if the basal cell should divide transversely. Both WOODBURN and HAUPT regard the foot as derived from the hypobasal cell, the stalk and capsule from the epibasal cell; the capsule being formed from the two anterior cells of the three derived from the epibasal cell, according to HAUPT. The sequence of the early divisions and the behavior of the hypo-

basal portion of the embryo would seem to warrant the writer's interpretation.

While it may be probable that the future sporogenous tissue is cut off from the capsule wall by the first periclinal walls which form in this portion of the embryo, it does not show differential staining until later, when the physiological differentiation becomes evident, as shown in the more massive capsule (fig. 46) of the winter condition. CAVERS (4) holds to the view that "the capsule wall in Marchantiales is not differentiated until a relatively late stage; that is, the separation of the archesporium is not determined by the first periclinal divisions in the young capsule." Further development takes place the succeeding spring, with the sporophyte reaching maturity, in this latitude from the middle of May to the middle of June. The writer has not made a careful study of sporogenesis, HAUPT'S paper giving an account of the features in detail.



FIGS. 42–47.—Figs. 42–44, transverse sections of embryos showing first periclinal walls; fig. 45, longitudinal section of young embryo late in November (note prominent basal cell); fig. 46, embryo in winter condition; ×350; fig. 47, base of embryo in winter condition (large basal cell quite conspicuous).

Calyptra and involucre

The calyptra grows apace as the embryo develops, becoming several layered and relatively somewhat massive. The longitudinal axis of both embryo and calyptra becomes more and more vertical, until finally it is practically perpendicular to the substratum, with the neck of the archegonium hanging downward. The calyptra incloses the sporophyte until spring, when the rapid growth of the latter breaks through the slower growing calyptra

and becomes exposed, excepting where covered by the receptacle tissue, which has grown downward and formed an involucre about both calyptra and sporophyte, dorsally and laterally.

Discussion

The finding of filamentous embryos in an increasing number of Marchantiales makes it evident that the octant type of embryo is not necessarily the rule in this group, in contrast with the filamentous embryo of the Jungermanniales. This, together with the occurrence of oblique walls and even a triangular apical cell, tends to bring the Marchantiales and Jungermanniales closer together as regards their embryogeny, and in an occasional partial agreement with that characteristic of the Musci.

In a previous paper, the writer (**6**) referred to the plasticity of *Reboulia* as shown by the male reproductive structures. The variations found in the development of the embryo give additional support to that view.

In the differentiation of the capsule region from the foot and stalk, *Reboulia* is probably like that of most Marchantiales, in that the capsule is generally derived from the epibasal half of the egg. Even in *Reboulia* there is no absolute proof that the epibasal cell may not contribute in part to the stalk region, as is the case in the Jungermanniales. The behavior of the basal cell of the filament is suggestive of an approach to the situation in some of the Jungermanniales where the entire hypobasal cell is a mere appendage to the embryo.

Summary

1. The mature egg and early embryo are elongated, slightly curved, bilaterally symmetrical bodies.

2. Fertilization takes place in October, the development of the embryo beginning at once, the sporogenous tissue becoming differentiated by winter, the sporophyte maturing in May and June.

3. The early embryo shows considerable variation in its development, the chief features being: (1) the first division of the egg may be transverse or oblique; (2) transverse division of both hypobasal and epibasal cells results in a filamentous embryo of four cells;

(3) vertical wall formation occurs in these four cells, with the exception, commonly, of the basal cell; (4) oblique walls may occur in any part of the embryo, and are not uncommonly to be found in the apical region where they may form a triangular apical cell, functional in the cutting off of a few segments; (5) the foot and stalk are probably derived from the hypobasal cell, the epibasal cell giving rise to the capsule, although it may conceivably make some contribution to the stalk as well; (6) the basal cell of the row of four varies in its contribution to the tissue of the foot, at times apparently remaining undivided, in which case the remainder of the foot and the stalk is derived from its sister cell.

4. The variations in the early embryo support the view that *Reboulia* is a plastic form, and as such may occupy a genetic position among the Hepaticae.

JUNIATA COLLEGE
HUNTINGDON, PA.

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SPECIFIC ACIDITY OF WATER EXTRACT AND OXALATE CONTENT OF FOLIAGE OF AFRICAN SORREL[1]

GEORGE PELHAM WALTON

(WITH ONE FIGURE)

Foreword

This report is submitted, not so much as a contribution to the accumulated data on the composition of the leaves of *Rumex abyssinicus* Jacq., as for the purpose of directing the attention of food and drug analysts to a comparatively simple procedure by which much of value may be learned about the source of the acidity in certain acid materials, with the minimum expenditure of time and effort. The scheme outlined in this paper has already been applied by the writer to a study of dried apple pomace and pectin pulp, with gratifying results, and it is believed that it should prove of value in the study of other feeding stuffs of an acid character.

Material examined

Among the plants brought to the United States for further study by the Office of Foreign Seed and Plant Introduction of the United States Department of Agriculture in 1919 was a native African sorrel, *Rumex abyssinicus* Jacquin.[2] The stock was brought from Angola, Portuguese West Africa, where the foliage is reported to be used as greens for human food. Dr. DAVID FAIRCHILD states as follows:

> Preliminary trials at various points in this country have shown this species to possess distinct promise as a summer vegetable. By sufficient parboiling or boiling in a large amount of water, its acidity can be reduced to a point where it is distinctly agreeable, and since it is devoid of all stringiness it deserves to be widely known in America. The plant grows to a height of eight feet and produces an amazing amount of greens throughout the summer. It is as resistant to heat as New Zealand spinach and Swiss chard.

Dr. ARNO VIEHOEVER suggests caution, however, in the use of this material for food. The following statement by him[3] was written after the completion of the chemical work reported in this paper.

[1] From the Cattle Food and Grain Investigation Laboratory, Bureau of Chemistry, United States Department of Agriculture, Washington.

[2] S.P.I. no. 48023.

[3] Amer. Food Jour., January 1922.

The presence of considerable amounts of oxalic acid, as found in the Bureau of Chemistry, in the form of soluble oxalates, as well as calcium oxalate, suggests caution. It is a well known fact that some people are especially susceptible to poisoning from oxalic acid, and the poisoning cases reported after eating rhubarb leaves are by no means rare. It appears possible, however, that by the addition of calcium carbonate the soluble oxalates may be precipitated, and thus the major portion of the objectionable ingredient may be eliminated. Another means suggested, and possibly equally effective to make the product available for general consumption, would be the removal of the water in which the material has been soaked and boiled. At any rate, we have here a product which may be placed on the market, and which may be used as a substitute for spinach and other greens, but the identity and characteristics of which should be known to the consuming public and especially the food officials concerned with the welfare of the people.

Several plants started in the vicinity of Washington in 1920 made vigorous, healthy growth (fig. 1). Because of the promising character of the plant as a source of summer greens, it was decided to submit a sample to the Bureau of Chemistry for a determination of its oxalic acid content. The suggestion was made, however, that in order to decide the question of its wholesomeness it was essential that both the total (titrable) acidity and specific acidity (H-ion concentration) of a water extract of the material be determined, as well as the total oxalate content. These determinations were undertaken in conjunction with the colorimetric determination of specific acidity in certain feeding stuffs.

TABLE I

DIMENSIONS OF LEAVES OF SAMPLES IN MM.

DIMENSION	SAMPLE NO. 38339			SAMPLE NO. 38340		
	Largest	Smallest	Average of 20	Largest	Smallest	Average of 11
Length of blade	195	75	149.0	260	230	249
Extreme breadth of blade	150	45	118.0	250	200	220
Length of petiole	120	40	86.5	140	100	125

On August 5, the plants in Washington being at a suitable stage of growth, a sample of about one pound of fresh foliage was analyzed. Practically all of the material obtained consisted of sound, crisp leaves with petioles. As there were two distinct sizes of leaves, the material was divided into two samples. The dimensions of the leaves constituting these samples are given in table I.

FIG. 1.—Row of plants of Abyssinian *Rumex*, about 6 feet high, grown at Yarrow Plant Introduction Garden near Rockville, Md. Photograph furnished by Office of Foreign Seed and Plant Introduction, Bureau of Plant Industry, U.S. Department of Agriculture.

Procedure

MOISTURE

The bulk of the material of each sample was used for the determination of moisture, but only entire leaves, with their petioles, were taken. After recording the total green weights, the midribs, larger veins, and petioles were split by a sharp knife to facilitate evaporation, and the material was rapidly dried at 65°–70° C. in a well ventilated oven, until friable. It was then crushed, care being taken to avoid loss of substance, and the samples finally dried to constant weight at 65°–70° C. in a vacuum oven. The loss in weight, about 90 per cent of the green weight, was taken to be total moisture. The dried material was ground and reserved for further study.

TOTAL ACIDITY OF WATER EXTRACT

Several sound leaves with petioles, representative of the fresh green material, were selected from each sample, and the weights of the two charges recorded. In the case of the larger foliage two leaves, weighing 43 gm., constituted the charge, while several leaves, weighing in the aggregate 25 gm., represented the sample of smaller foliage. Each charge was thoroughly macerated in a glass mortar, and the resulting pulp transferred to a four-sided glass 8 oz. sample jar, with exactly 200 cc. of distilled water, previously boiled and cooled to room temperature. After violently stirring the mixture for 30 minutes by means of an electric mixer,[4] it was thrown on dry filter paper. The first (cloudy) portion of the filtrate was rejected, and the total acidity in an aliquot of the clear extract was determined by titrating with N/10 sodium hydroxide solution, using phenolphthalein as indicator. The presence in the water extract of a natural indicator,[5] the strongly darkening color of which tended to obscure the end-point of the titration, made it necessary to carry through concurrently a blank with a similar aliquot of extract with-

[4] Described in Circular 68, Office of the Secretary, U.S. Department of Agriculture.

[5] This natural dye in the leaves of *R. abyssinicus* Jacq. appears pink in dilute aqueous extracts of natural acidity. As titration with a fixed base progresses, the color changes through yellow to brown, at about the neutral point, and the solution becomes inky when made distinctly alkaline.

out phenolphthalein. In this blank titration, the addition of N/10 sodium hydroxide solution kept pace with the quantity added in the true titration, and the end-point was determined by contrasting the colors of the titrated extracts. The extract containing phenolphthalein developed a noticeably redder brown color than the other.

In the computations allowance was made for the water present in the green material. The total titrable acidity is expressed as cubic centimeters of normal acid per kilogram of leaf material, termed the "degree of acidity."

Specific acidity of water extract

The specific acidity was estimated on a portion of the clear extract by a slight modification of the colorimetric method described by Gillespie (3) after Barnett and Chapman (1), in which use is made of the principle introduced by Clark and Lubs (2), following Salm (7), of "superimposing the two extreme colors of an indicator in determining its half-transformation point." Instead of using a system of nine pairs of tubes having drop ratios 1:9, 2:8, etc., Medalia's (4) system of seven pairs, having P_H exponent intervals of 0.2 between each pair for the indicators used, was adopted. For convenience the procedure is briefly sketched here.

The color comparisons are made in the small "block" comparator described by Gillespie. Seven pairs of test-tubes, selected to fit the comparator and for their uniformity in bore, are calibrated for 5 cc. capacity and arranged in a double row test-tube rack. A total of eight drops of the suitable indicator solution is delivered into each pair of tubes, 1 to 7 drops in the front seven tubes and 7 to 1 drops in the back row, care being taken to hold the delivery pipette in an upright position. Sufficient alkali[6] (dilute acid in the case of the indicator thymol blue, acid range) is then added to the tubes in the front row to produce the full alkaline color, and sufficient acid to develop the full acid color is added to those in the rear row. The tubes are then carefully filled to the 5 cc. mark with distilled water, previously boiled and cooled. Similar tubes are used for the solutions under examination (the water extracts of the sorrel). Eight drops of the indicator solution are required, of course, and the

[6] Quantity of alkali or acid varies somewhat for the different indicators.

5 cc. volume is completed with the "unknown" solution. The contents of all tubes are well mixed before making the color comparisons. (Mixing may be accomplished by rolling the tube back and forth between the palms of the hands.)

In making the color comparisons, the tubes, held vertically in the comparator, are arranged in two files of three tubes each, one file being made up of the tube containing the "unknown," *with* indicator solution and two tubes of distilled water, and the other file consisting of a pair of the standard tubes and a tube containing the "unknown" solution, *without* indicator. This arrangement is necessary to obviate optical differences caused by thickness of liquid viewed on the one hand, and on the other to offset the natural color and any turbidity of the extract under examination. Different pairs of standards are tried until the color of light passing horizontally through that file of tubes matches the color from the file containing the tube of "unknown," *with* indicator.

As stated by GILLESPIE, the tubes are viewed best against the sky. Occasionally, in the case of certain indicators, such as bromphenol blue, trouble is experienced in matching the colors because of a dichroic effect, especially noticeable in turbid solutions. In such cases the tubes may be viewed by the yellow light of a carbon electric lamp, screened as advised by CLARK and LUBS. Only two indicator solutions were needed in estimating the specific acidity of the sorrel extracts, an 0.05 per cent aqueous solution of bromphenol blue,[7] and an 0.02 per cent solution of thymol blue (thymolsulphonphthalein) in 80 per cent alcohol.

To develop the full acid and "alkaline" colors respectively, in the standard paired tubes, the following quantities of reagents were used for the two indicators:

Bromphenol blue.—To produce the acid color, 0.5 cc. of N/10 hydrochloric acid solution; to produce the alkaline color, 1 drop of N/20 sodium hydroxide solution.

Thymol blue (acid range).—To produce the full acid color, 2 cc. of 1.25 per cent hydrochloric acid solution; to produce the color of

[7] Tetrabromophenolsulphonphthalein. The 0.05 per cent solution was prepared by diluting one volume of the indicator solution, furnished in the LaMotte field set, to twenty volumes, with freshly boiled and cooled distilled water.

the "alkaline" end of the range, 1 cc. of 0.005 per cent (N/700) hydrochloric acid solution.

The volume in all the tubes should be made up at once to 5 cc. The specific acidity values accepted for the several drop ratios are given in table II. This specific acidity is based on the H-ion concentration of pure neutral water as unity, as defined by WHERRY (10). The articles by WHERRY and ADAMS (11) and CLARK (11) discuss this system of stating H-ion concentration.

TABLE II

SPECIFIC ACIDITY VALUES IN ROUND NUMBERS

DROP RATIO	BROMPHENOL BLUE		THYMOL BLUE, ACID RANGE	
	Specific acidity	P_H	Specific acidity	P_H
1:7	6300	3.2	400,000	1.4
2:6	4000	3.4	250,000	1.6
3:5	2500	3.6	160,000	1.8
4:4	1600	3.8	100,000	2.0
5:3	1000	4.0	63,000	2.2
6:2	630	4.2	40,000	2.4
7:1	400	4.4	25,000	2.6

These specific acidity (and P_H) values, while not absolutely exact, are close enough for the purposes of this investigation, particularly as it was found that the specific acidity of the sorrel extracts fell at the extreme acid end of the bromphenol blue series, or between that and the "alkaline" end of the thymol blue, acid range, where a close estimation is impossible.[8] The sorrel extracts, however, were checked up by comparison with the straight acid color of the first indicator and the straight "alkaline" end color of the thymol blue, acid range.

The color standards are quite permanent, and if the tubes are stoppered and kept in the dark they may be used over a long period (4). The literature citations, particularly 3 and 4, contain details on the use of indicators covering P_H values from 1.2 to 9.8. Both titrable and specific acidity were determined also on water

[8] As stated by GILLESPIE, measurements cannot be accepted at the point where the drop ratio is 9:1 or 1:9 (7:1 or 1:7), as the percentage transformation of the indicator is so nearly 100 or zero that the H^+ exponent may be far from that represented by the ratio, and this would not be disclosed by a difference in color.

extracts of dried and ground material of both samples. All determinations were made at room temperature, 25°–30° C. The higher temperature was usually reached in the afternoon during August.

Total oxalate

The estimation of total oxalate was undertaken for the purpose of verifying the figures obtained in the acidity work, and a critical study of oxalate methods was not attempted. After the completion of this work, the writer's attention was directed to a method, perfected by W. F. Kunke, Bureau of Chemistry, for the determination, with a high degree of accuracy, of the total oxalate content of plant material. Using this method on another sample of the sorrel foliage, Kunke obtained materially lower figures than those reported in this paper for total oxalate. Because of this and the relative crudity of the usual method, the figures for total oxalate herein reported are probably somewhat high. This, however, in no way invalidates the data dependent on the acidity determinations (the figures for potassium binoxalate), as whatever error there may have been appears in the figures for calcium oxalate.

The total oxalate content was estimated only on the dried and ground material. Two gm. was weighed into a 150 cc. volumetric flask, about 100 cc. of 2 per cent hydrochloric acid solution added, and the mixture, after being heated to boiling, was digested for thirty minutes on the steam bath. After cooling and completing the volume to 150 cc. with distilled water and mixing, the extract was filtered through dry filter paper. The total oxalate in 100 cc. of the hydrochloric acid extract was estimated by precipitation with calcium chloride and titration of the oxalate with standard solution of potassium permanganate, in the usual way. Contamination of the calcium oxalate precipitate with organic matter necessitated double precipitation, and the final precipitate was washed with 1 per cent acetic acid solution in the cold, for further purification.[9] Two control determinations on pure sodium oxalate were conducted under the same conditions as those for the sorrel samples. In the titration, which was carried through rapidly, there appeared to be a

[9] Final precipitation of calcium oxalate in acetic acid solution would be preferable for a material of this nature.

definite end-point, at which the pink color of the permanganate persisted for an appreciable interval of time, although organic matter, small amounts of which undoubtedly were present, continued slowly to reduce additional permanganate.

Results

The data obtained in the chemical examination of the fresh and dried sorrel leaves are given in tables III, IV, and V.

TABLE III

ANALYSIS OF LEAVES OF *Rumex abyssinicus*

SAMPLE	PERCENTAGE MOISTURE AT 65°–70° C.	DEGREE OF ACIDITY (cc. normal acid per kg.)	PERCENTAGE TOTAL OXALATE AS (C_2O_4)*
Misc. Div. No. 38339, small leaves with petioles (fresh)	90.77	152.7	1.69
Dried at 65°–70° C. and ground	2.99	1490.0†	17.8
Misc. Div. No. 38340, large leaves with petioles (fresh)	89.59	185.4	1.73
Dried at 65°–70° C. and ground	2.50	1500.0†	16.2

* As stated previously, according to KUNKE these figures may be somewhat high.

† Evidently there was a decrease in acidity during drying, for the degrees of acidity as determined on the dried material, computed to the original moisture bases, would be 141.8 and 160.2 respectively.

TABLE IV

WATER EXTRACT OF LEAVES

SAMPLE	QUANTITY OF SAMPLE PER 100 CC. OF EXTRACT (gm.)	TOTAL ACIDITY IN 100 CC. OF EXTRACT EXPRESSED AS NORMAL ACID (cc.)	SPECIFIC ACIDITY, H-ION CONCENTRATION			
			Of water extract of *Rumex* leaves (observed)		Of aqueous solution of HKC_2O_4 having same total acidity as water extract of *Rumex* leaves (computed)*	
			Specific acidity	P_H	Specific acidity	P_H
No. 38339, small leaves with petioles (fresh)	11.26	1.72	6300–10,000	3.2–3.0	9000	3.05
Dried	1.00	1.49	6300	3.2	8400	3.08
No. 38340, large leaves with petioles (fresh)	18.07	3.35	10,000–16,000	3.0–2.8	12600	2.90
Dried	1.00	1.50	6300	3.2	8400	3.08

* The method of computation is discussed later under Discussion. Attention is directed to the close agreement between the observed values and those computed for a pure potassium binoxalate solution.

Table IV gives the figures relating to the acidity of water extracts of both the fresh and dried material, and specific acidity values computed for pure solutions of potassium binoxalate of the same respective normalities (titrable acidities) as the water extracts.

TABLE V

PERCENTAGE OF OXALATES AND EQUIVALENTS IN LEAVES

Sample	HKC_2O_4 in leaves (based on acidity of water extract of dried material)	(C_2O_4) equivalent of HKC_2O_4 in preceding column	Total (C_2O_4) in leaves from table III	Total (C_2O_4) minus acid (C_2O_4), column 4 minus column 3	Calcium oxalate (CaC_2O_4) in leaves equivalent of (C_2O_4) in preceding column
No. 38339, small leaves (fresh)	1.82	1.25	1.69	0.44	0.64
Dried	19.09	13.10	17.80	4.70	6.80
No. 38340, large leaves (fresh)	2.05	1.41	1.73	0.32	0.47
Dried	19.22	13.20	16.20	3.00	4.40

The data in table V are derived entirely from determinations made on the dried material. In the second and last columns the data from tables III and IV are correlated to show the percentage amounts of salts of oxalic acid presumably present in both the fresh and dried leaves. The percentages given for calcium oxalate are for the anhydrous salt, for convenience in comparing with data in pharmacological literature.

The assumption that most of the oxalate is present as potassium binoxalate and calcium oxalate (as the monohydrate) is substantiated by additional information obtained through the kindness of DR. WHERRY. The work of MR. DEUEL on total soluble oxalate also checks in a striking manner the figures for binoxalate.

CRYSTALLOGRAPHIC-OPTICAL EXAMINATION

WHERRY, who examined some of the dried and ground material by polarized light, under a petrographic microscope, identified numerous crystals of potassium binoxalate, and a smaller number of crystals of calcium oxalate monohydrate in groups. The potassium binoxalate crystals were readily identified, because of their characteristic of having a relatively low alpha index. WHERRY

states that four substances might conceivably be present in the sample of plant tissue, and first determined their optical properties as follows:

	Alpha	Beta	Gamma	2 E.	Sign
Oxalic acid dihydrate	1.445	1.505	1.540	120°	−
Potassium binoxalate	1.415	1.545	1.565	65°	−
Potassium oxalate monohydrate	1.440	1.485	1.550	160°	+
Calcium oxalate monohydrate	1.490	1.555	1.650	Over 180°	+

Examination of the sample showed two distinctly different crystalline substances to be present, one in rosettes of acute crystals, the other in nearly equant grains. The first proved to have the refractive indices characteristic of calcium oxalate as indicated, the second to have those of potassium binoxalate. The calcium salt occurs as aggregates of crystals, and therefore looks more prominent, but considering the large number of small grains of the potassium salt which are scattered around, it is evident that the potassium salt is present in the greater amount. Immersion liquids 1.490 and 1.565 are most suitable for distinguishing the two, the potassium salt having one index much lower than the first, the calcium salt one much greater than the second. As no crystals were found to be present with gamma less than 1.560, or beta less than 1.540, it is to be inferred that neither oxalic acid nor neutral potassium oxalate is present, at least in significant amounts.

Total dissolved oxalates

Deuel has kindly consented to the inclusion in this paper of figures obtained by him for the soluble oxalate in a sample of foliage from the same group of plants from which these samples were obtained. His procedure was to boil the finely minced leaves in water for 1.5 hours, filter, make up the extract to definite volume, and in an aliquot determine the total soluble oxalate gravimetrically by precipitation as calcium oxalate, and after ignition, weighing as calcium oxid. In the two samples of foliage examined he found oxalate equivalent to 2.06 and 1.96 per cent potassium binoxalate. The percentage of moisture on the samples was not reported, but the figures are for the fresh leaves.

Discussion

Much may be learned about acid material of the same general type as the foliage of *Rumex* sp., merely from a determination of the total (or titrable) and the specific acidity of a water extract of it. By "same general type" is meant here acid material in which, as in the sorrel, the "acidity" is due almost entirely to forms of a single acid. If, in addition to total and specific acidity, the total amount of the acid radical present in the water extract and in the material itself be known, it is possible to draw fairly accurate conclusions as to the quantities of the several salts of that acid actually present, without making elaborate determinations of the several basic elements. Auxiliary use of the petrographic microscope may afford valuable qualitative substantiation of the conclusions.

In this investigation the mere determination of "degree of acidity" (total titrable acidity of the water extract) of the sorrel foliage meant little; the acidity might have been caused by the presence of free oxalic acid. Determination of the specific acidity (H-ion concentration), however, proved conclusively that the acidity of the water solution could not have been caused by the presence of the free acid; as for the normality involved, the specific acidity value of oxalic acid would have been approximately ten times[10] the values actually found. On the other hand, the agreement between the "specific acidity" values determined experimentally for the sorrel extracts and those computed for pure solutions of potassium binoxalate of the same normality is striking. These values for potassium binoxalate solutions were computed by the help of the formula: Percentage ionization $= 100\left(\sqrt{KV} - \frac{KV}{2}\right)$,[11]

[10] Computed from data reported by THOMAS (9) after OSTWALD (5). Oxalic acid of a dilution comparable with the water extract of sample No. 38339 (N×0.0172) is highly dissociated, the percentage ionized being 88.4 at 25° C., according to THOMAS' table.

[11] By an evident typographical error this formula in THOMAS' article was incorrectly stated: "Per cent ionization $= 100\sqrt{KV} - \frac{KV}{2}$." The method of calculating the P_H value is here appended in more detail, using as an example the data for the potassium binoxalate solution corresponding to the extract of the larger leaves: Normal-

derived from OSTWALD's dilution law, as explained in detail by THOMAS (**9**). In this formula V = the volume in liters, in which one gram molecular weight of the substance is dissolved, and K = the dissociation constant. The values used for K are those given in SCUDDER's (**8**) tables, and are for 25° C.

The data from the acidity determinations, therefore, point to potassium binoxalate as the sole source of the acidity of the sorrel extracts, at least of those of the dried material. Furthermore, a calculation of the percentages of this salt present in the samples based on the titrable "acid" in the extracts of the dried leaves yields figures agreeing very closely with those obtained by DEUEL for the oxalate (as potassium binoxalate) dissolved by boiling water.

These data, per se, do not preclude the possibility of the presence in the foliage of approximately equivalent quantities of free oxalic acid and normal potassium oxalate, which would simulate the acid salt, and, in fact, in aqueous solution would be identical with it. WHERRY's observations on the dried material decides the point beyond a doubt. The acid nature of the leaves is unquestionably due to the presence of potassium binoxalate.

On recalculating the figures obtained for titrable acidity in the dried material (on which are based the figures for potassium binoxalate in the leaves) to the original (green) moisture bases, it becomes apparent that there is a loss of titrable acid during the drying. These figures (footnote, table III) become 141.8 and 160.2 for "degrees of acidity" on the original bases, respectively equivalent to 1.82 and 2.05 per cent potassium binoxalate, while the acidity actually titrated in the fresh material yielded the figures 152.7 and 185.4 (in terms of potassium binoxalate corresponding to 1.96 and 2.38 per cent); therefore 10.9 and 25.2 cc. respectively of normal acid per kilogram of fresh leaves disappeared during the drying of the two samples. This lost "acid" may have been carbon dioxide or other weak volatile acids, or may be accounted for in part by changes in colloidal, acid-reacting protein. The discussion by

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ity of solution = 0.0335 N; hence $V = 29.85$, $K = 4.9 \times 10^{-5}$. Percentage ionization = 3.75+. H per liter = 0.0335 × 1.008 gm. H^+ per liter = 0.0375 × 0.0335 × 1.008 = 0.001267 = 1.267×10^{-3} ∴ P_H = algebraic sum of −3, and log. 1.267 = −2.897. Omitting the negative sign, $P_H = 2.9$ (specific acidity = 12670).

PFEFFER (6) of decrease in acidity in plant tissues (in life) and in sap, due to a rise in temperature (from 15° to 45°) and to exposure to sunlight, fully explains a loss in acidity of this magnitude, on drying fresh material in which the cells are still functioning.

From the figures obtained for total oxalate it becomes apparent that there is more oxalate present in the leaves than is accounted for by the potassium binoxalate. Further, this excess oxalate must be either insoluble or neutral in reaction if soluble. Again the crystallographic-optical examination made by WHERRY decides the point. The only normal oxalate found was the insoluble calcium salt. A recapitulation of analytical results is presented in table VI.

TABLE VI

ANALYSIS OF LEAVES OF *Rumex abyssinicus*

Leaves	Percentage moisture at 65°–70° C.	Percentage potassium binoxalate (HKC_2O_4)	Percentage calcium oxalate monohydrate ($CaC_2O_4 \cdot H_2O$)	Degree of acidity (cc. normal acid per kilo)
Smaller leaves (fresh).....	90.77	1.82	0.73	152.7
Larger leaves (fresh).....	89.59	2.05	0.53	185.4
Smaller leaves (after drying).	2.99	19.09	7.80	1490.0
Larger leaves (after drying).	2.50	19.22	4.98	1500.0

A discussion of the influence of such quantities of oxalate on the edibility of the sorrel foliage, or of the physiological effects following its use as food, is outside the scope of this paper.

Summary

1. The study here reported of the acidity and oxalate content of the leaves of *Rumex abyssinicus* (an African sorrel) demonstrates the advantages of determining the specific acidity (H^+ concentration), as well as the total (titrable) acidity of a water extract of acid material of this nature.

2. This paper brings together descriptions of relatively simple procedures, worked out by the investigators cited, for (1) colorimetrically determining, without the use of buffer solutions, the specific acidity of such water extracts, and (2) computing, for purposes of comparison, the specific acidity and P_H value of pure solutions of the acid substance suspected of being the source of the

acidity. Through a comparison of these values, matching the specific acidity actually determined against that computed for those substances causing the acidity, a means of identifying, or at least indicating, the principal source of the acidity is described.

3. The data indicate that only two compounds of oxalic acid, potassium binoxalate and calcium oxalate monohydrate, occur in the *Rumex* leaves examined. The percentages in which these salts occur are computed from the data for acidity and total oxalate in the dried material.

4. Attention is directed to the value of a crystallographic examination in corroborating the results of the chemical work. It is believed that the scheme of investigation described should prove of value to analysts in examining drugs, foods, or feeding stuffs of an acid character.

5. The presence of a natural indicator in the leaves of *R. abyssinicus*, the aqueous solutions of which are pink in the natural acid solution, is noted. On adding a fixed alkali the solution changes its color through yellow to brown, becoming nearly black when distinctly alkaline.

The writer wishes to thank Mr. PAUL G. RUSSELL of the Office of Foreign Seed and Plant Introduction for making examination of this material possible, for furnishing information as to the history of the plant and its culture in this locality, and for providing the fresh foliage for analysis. Also acknowledgment is due Messrs. DEUEL and KUNKE for their kindness in permitting the inclusion of notes of their work, and Dr. WHERRY for his helpful suggestions and for the crystallographic examination of the leaf material.

BUREAU OF CHEMISTRY
WASHINGTON, D.C.

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MICROBIOLOGY OF FLAX RETTING

FRED W. TANNER

Linum usitatissimum has been cultivated for thousands of years as a textile fiber producing plant. The Egyptians must have raised it, since their mummies are found today wrapped in fine linen. Frequent allusions to flax and linen in the Bible indicate that the ancients were acquainted with the usefulness of the bast fibers in flax and had methods of separating them from the rest of the plant. They were also familiar with other types of fibers, since these are found in their papyri today. The United States cannot be regarded as a great flax or linen producing country; it has had to depend mainly on importation to supply the increasing demands for linen. In the spinning of flax the United States was at the bottom of the list of the larger countries in 1915, with slightly over 8000 spindles against Great Britain's 1,161,000.[1] Most of these were in Ireland, although they were not kept busy on fiber produced in Ireland. Russia was once the largest flax fiber producing country, contributing 80 per cent of the flax fiber used in making linen. Since the world war, however, this has changed on account of the industrial disorganization in that country. Statements in the press, said to come from the Office of Fiber Investigation of the United States Department of Agriculture, indicate that the spinning mills in this country have used about 10,000 pounds of flax fiber per annum. For the production of this amount of fiber about 60,000 acres of land would be required. In 1920 only 6000 acres of flax were grown in this country, while the low price paid for it will restrict the acreage to about 3000 in the future.

Flax is raised in this country mostly for seed which is pressed for linseed oil; a smaller amount is raised for the fiber. Flax raised for seed is of a different quality from that usually required for fiber. Fiber flax is taller and produces less seed. It requires greater care in cultivation, and especially careful handling at the

[1] MILES, C. F., Fiber flax. U.S. Dept. Agric. Farmer's Bull. no. 669. 1915.

harvest. Some claim that it must be pulled, not cut, and tied up carefully in bundles. This may be one reason why it has been difficult to utilize the flax from seed flax for spinning. It might be possible in the future to combine profitably the seed and fiber crop. This would tend to reduce the value of each crop taken by itself perhaps, but the value of the combined crops of seed and fiber might compensate for any decrease in the value of the single crop.

The bast fibers, which are those used in making linen, are cemented to the other parts of the stalk and to each other by means of materials, for convenience, called pectins. Undoubtedly this term is used only in a general way to cover a number of compounds closely related chemically. The aim of the retting process is to remove these "binders" without harming the cellulose fiber. The fermentation must be checked when these fibers have been freed by the hydrolysis of the pectose or salts of pectic acid. These binding materials which hold the stalk together are undoubtedly carbohydrate in nature, and thus susceptible to the action of microoganisms.

Preparation of flax fiber

The fiber is prepared from the flax straw by a special process which seems to have been built up after a long period of time without much assistance from the sciences. Proper harvesting is very important. Fiber flax should be pulled either by hand or by machinery and tied into bundles which are shocked for curing. Cutting the flax is claimed by some to leave the ends of the stalk exposed for undesirable decompositions. When the heads are shocked for curing, this cut end becomes susceptible to the attacks of undesirable bacteria. The fibers become badly stained also. This may not be entirely true, however, under actual practice.

After curing, the stalks are retted. This is really a rotting process, which indicates the origin of our present term. Three general methods may be used to dissolve the binder which holds the cellulose fibers to the woody materials: dew retting, water retting, and chemical retting. The first two only are of bacteriological interest, and were studied in the present investigation.

Dew retting was used by our forefathers in this country for preparing flax fiber for spinning. It represents the earliest method of preparing flax fiber. No special apparatus is needed, since the flax straw is merely spread on the ground in the fall and allowed to remain throughout the winter. Dew retting has been used for the preparation of most Russian flax fiber. Its greatest objection is the time required, but this may be reduced greatly by carrying the process out under conditions where the retting organisms may be made to work harder.

Water retting was introduced undoubtedly to get away from certain of the distinct disadvantages of dew retting. It is carried out either in slow flowing rivers or in ponds and other inclosed bodies of water. The bundles of flax straw are packed into these basins and weighted down. The retting process starts with a gaseous fermentation of the carbohydrate materials in the flax straw. If conditions are favorable, a little over ten days is necessary for the completion of the fermentation. The flax should be removed when all the pectic materials are dissolved, or over-retting will result. The bundles are removed, dried in sun and air, and are then ready for scutching. The river Lys in Belgium is famous for its flax retting. River retting has certain economic features which limit its wide application. As KUHNERT[2] has shown, the stream becomes putrescible, which is detrimental to fish life. It carries amounts of organic materials in the reduced conditions which may give off objectionable odors. Water retting has not had wide application in this country.

Several attempts have been made to improve on the water retting. One of the earliest of these was proposed by SCHENCK in 1846. The flax straw was packed tightly into a tank and the water kept at a temperature of 75°–95° F. This warm environment was more favorable to the development of the bacteria concerned in this process, and a vigorous fermentation quickly established itself. The vats had all of the characteristics of a fermentation mixture. Others have proposed similar methods with a higher temperature.

Scutching is the process by which the woody material is broken away from the cellulose fibers after they have been retted and

[2] KUHNERT, Landw. Wochenbl. Schleswig-Holstein **70**:540–543. 1920.

dried. Different methods have been used, all of which rest on breaking the woody particles and mechanically removing them from the stalk. The fibers are finally combed to separate the "tow" from the fibers which are not long enough to remain in line. The latter may be used in paper, coarse linen, etc. The fiber from flax may be 30–40 inches in length, thus yielding a product which is valuable for spinning.

Microbiology of retting

Retting is indeed a natural process, and may be regarded as merely a step in the cycles of the elements. The various factors involved have been separated in an attempt to intensify certain ones in order to make the process shorter, and also to produce a better fiber. In retting flax man has simply made use of and intensified a reaction which is always going on.

One of the first investigations on the microbiology of retting was carried out by VAN TIEGHEM[3] in 1879, in his study of the process of water retting. An anaerobic organism named *Bacillus amylobacter* was reported as the organism which quickly decomposed the pectic materials of the flax stalk. In the same year VAN TIEGHEM stated that his *Bacillus amylobacter* was probably identical with the *Vibrion butyrique*. An aerobic spore-forming organism was also found by WINOGRADSKY.[4] FRIBES, working in this laboratory, tried various disinfectants for sterilizing the flax, but finally used the method of heating in water at 100° C. for three successive days, or at 115° C. for fifteen minutes. Various aerobes and anaerobes were isolated, none of which seemed to have any effects on the flax. Finally a specific anaerobe was isolated. It was a spore-former, the young cells of which were 10μ to 15μ by 0.8μ. Glucose, sucrose, starch, and lactose were fermented if some nitrogenous matter was present. A quite similar microorganism was also isolated by BEHRENS[5] from the water retting of hemp. The organism was a *Clostridium* form fermenting the binding materials of hemp straw.

[3] VAN TIEGHEM, Sur la fermentation de la cellulose. Bull. Soc. Bot. France **26**:25. 1879.

[4] WINOGRADSKY, S., Sur le rouissage du lin et son agent Microbien. Comp. Rend. Acad. Sci. **121**:742. 1895.

[5] BEHRENS, J., Cent. Bakt. **10**:524. 1903.

It fermented glucose, sucrose, fructose, lactose, galactose, and starch with abundant gas formation, but could not attack arabinose, cellulose, gum arabic, or calcium lactate. Similar to WINOGRADSKY'S organism, it required some source of nitrogen, as peptones or proteins. It was an obligate anaerobe with large spores which had a greater diameter than the vegetative rod.

In 1902 HAUMANN[6] published an interesting paper which gave an entirely new aspect to the subject. He stated that many common microorganisms could ret flax. He first studied the flora on the stalks of retted flax and isolated a number of organisms, among which were *Bacterium coli-communis*, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Streptothrix Forsteri*, *Penicillum glaucum*, *Cladosporium herbarum*, *Bacillus mesentericus fuscus*, *B. mycoides*, *B. termo*, *Micrococcus roseus*, and *Mucor mucedo*. The mere presence of these organisms would not indicate that they functioned in retting. The preponderance of certain species, however, might indicate some relation to the retting process. *Cladosporium herbarum*, *Bacillus mesentericus*, *B. subtilis*, and colonies of *Streptothrix* were common. To determine whether these organisms were important, HAUMANN inoculated sterile flax with pure cultures. The flax stalks were put into long culture tubes plugged with cotton. The tubes containing flax were heated to temperatures below 110° C. in the dry condition. He stated that three heatings under such conditions did not alter the flax. Retting was accomplished by using many of the common species of microorganisms. There was a difference in action, *Pseudomonas fluorescens* giving good results, while *Micrococcus roseus* was least satisfactory. HAUMANN concluded from this that all of the common bacteria were able to ret flax. Some of these bacteria were also able to split pectin. His results are unique in that they contradict those secured by many others and also those obtained in this investigation. In the light of some of the recent work on thermal resistance of the spores of anaerobic bacilli, HAUMANN'S method of sterilizing the flax is open to criticism. One would not expect these spores to be destroyed by a temperature of 110°, especially in the dry conditions. It is

[6] HAUMANN, M. L., Étude microbiologique et chimique du rouissage aerobic du lin. Annals Past. Inst. **16**:378–385. 1902.

possible that some of the spores of anaerobic bacteria survived and produced the characteristic change in the flax fiber which was attributed to the pure culture of aerobes used.

An anaerobic organism was also found by BEIJERINCK and VAN DELDEN,[7] to which they gave the name *Granulobacter pectinovorum*. This organism was an obligate anaerobe and a vigorous spore-former. It required protein or its split products as sources of nitrogen, liquefied gelatin, and actively attacked carbohydrates. In general it had the characteristics of the anaerobes described by others, especially the one found by WINOGRADSKY.

A more recent extensive investigation has been reported by STORMER,[8] who found an anaerobic spore-former which he called *Plectridium pectinovorum*. The granular structure of the cells makes one believe that he had the organism described by earlier writers. STORMER's bacillus seemed to differ from these, however, in being a facultative anaerobic organism.

In sharp contrast with these papers are a number of others indicating that an aerobic organism is involved. MARMIER[9] mentioned such an organism. BEIJERINCK and VAN DELDEN also reported that *Bacillus subtilis* and *B. mesentericus* would ret flax, although they found another organism, which they called *Granulobacter pectinovorum*, which seemed to ret flax more completely and quickly.

ROSSI[10] studied the retting of vegetable fibers and stated that the microbiological retting process has certain advantages over chemical retting. Whether this is true or not is probably determined by the uses to which the fiber will be put. He devised an aerobic method in which *Bacillus comesii* was allowed to act on material which had been steeped in water at from 28° to 35° C. The pure culture was added and the vat was aerated, the tempera-

[7] BEIJERINCK, M. W., and VAN DELDEN, A., Over de bacterien welke bij roten van vlas werksdam zign. Kon. Ak. Wetensch. Amsterdam (Verstag. van de Gewone Vergadering der Wis en Natunkk) **12**:673. 1903.

[8] STORMER, K., Über die Wasserroste des Flachses. Chem. Zent. **76**:41. 1905.

[9] MARMIER, Le rouissage du lin. Bot. Centrabl. **83**:90. 1900.

[10] ROSSI, G., Industrial retting of textile plants by microbiological action. Bull. Agric. Intell. **8**:1067–1074. 1916.

ture being maintained between the extremes just mentioned, since the fermentation goes on most vigorously at that range. ROSSI's work was concerned with hemp, but the organisms may be similar to those used with flax. An anonymous article in the *Bulletin Imperial Institute* (**17**:605–607. 1919) confirms the work of ROSSI. Flax straw is immersed in water at 82° to 86° F. in vats, and after the addition of a special aerobic organism, *B. comesii*, it is aerated during the retting process. The pectinous materials are consumed and the retting process is completed in 36–40 hours. Another aerobic organism called *Bacillus felsineus* is reported which will also ret hemp, flax, ramie, nettle, and other plants. It is said to produce a rapid retting and furnish a fine, white, well separated fiber. LOESER,[11] in carrying out ROSSI's process, boiled flax for forty minutes and then treated it with pure cultures of *Bacillus comesii*. The vats were aerated and maintained at a temperature of 30°–32° C. These methods are more expensive than those which do not require boiling. Furthermore, the anaerobic organisms are so resistant to heat that they might pass through these preheating procedures and function in retting the flax, although the credit be given to certain aerobic bacteria.

CORRIGAN[12] has more recently discussed the relation of fungi to retting. His statements apparently are based on the researches which have been reviewed herein. Mrs. WYANT[13] has recently reported briefly some results on this problem. She isolated about forty cultures of both aerobes and anaerobes. Each of these cultures was tested for its retting ability. This narrowed the work down to one pure culture which received more intensive study.

Experimental work

The work which has been carried out at this laboratory has been done with pure cultures, and on materials from a large rettery

[11] LOESER, R., Retting of flax by means of bacteria. Jour. Soc. Chem. Ind. **38**:407. 1919.

[12] CORRIGAN, J. FREDERICK, Bacteria and molds: their biological nature and their influence on vegetable fibers. Jour. Soc. Dyers Colourists **36**:198–201. 1920.

[13] WYANT, Z. N., Some bacteriological problems involved in the retting of flax. Abstracts Bact. **5**:208, 209. 1921.

in eastern Michigan.[14] Most of the flax used in these experiments was grown in Michigan, and was thoroughly cured before it was received at the laboratory. It was tied in bundles or "heads" and was in fine condition, since it had been cultivated for fiber. The other was the flax which had been raised for seed, and consequently had not been kept tied in bundles, but had become badly broken and bent during thrashing. While just as good retting was secured on this as with the stalks which were raised for the fiber and tied in bundles, the resulting fiber was not of good quality. Perhaps such a raw material could be better retted by the chemical process. The fibers were much shorter, but probably just as satisfactory for paper making.

The samples from the retting vats were taken in sterile bottles and subjected to the usual bacteriological examination. Both aerobic and anaerobic plates were made, from which pure cultures were picked and transplanted into various culture media. The flora from the vats was varied, but spore-forming bacteria of both aerobic and anaerobic types were common. The aerobic types were similar to the members of the *subtilis-mesentericus* group; they formed large spreading colonies on solid media and liquefied gelatin very rapidly. Spores were easily formed in large numbers.

The pure culture experiments were carried out with many of the common bacteria and several cultures of yeasts. The bacteria and yeasts used were:

Pseudomonas pyocyaneus	Saccharomyces cerevisiae
Proteus vulgaris	Torula monosa
Erythrobacterium prodigiosus	Saccharomyces ellipsoideus
Pseudomonas fluorescens	Saccharomyces marxianus
Zopfii Zenkerii	Myoderma vini
Bacterium enteritidis	Bacterium cloacae
Bacillus gasoformans	Bacillus cereus
Bacterium colon	Bacterium capsulatum
Bacillus butyricus	Erythrobacillus arborescens
Bacterium aerogenes	Bacillus subtilis

[14] The author is indebted to Mr. B. S. SUMMERS of the Summers Linen Company of Port Huron, Michigan, for material upon which some of the results here reported were secured, and for his constant interest in the progress of the work. Dr. R. E. FINDFUSZ of the American Writing Paper Company kindly furnished some flax straw and exhibited a kindly interest.

Contrary to the statements of HAUMANN, successful retting could not be accomplished with these common bacteria. None of the organisms such as *Bacillus subtilis* was found to possess any activity. In order to test the retting ability of all these cultures and those isolated from vat liquors, flax straw was cut into pieces about 4.5 inches long. These were put into long test tubes and covered with water. Sterilization was accomplished by heating in the autoclave at 115° C. for fifteen minutes. The sterility of these flax straw culture tubes was determined by both aerobic and anaerobic cultures in sterile litmus milk and other media. Heating in the autoclave at 115° C. seemed to be sufficient for their sterilization, and did not seem to injure the straw or make the retting more quickly accomplished when the bacteria were applied. Experiments were carried out later in 3 gallon earthenware jars in order to test the retting activity of the organism which was finally isolated. Attempts were also made to find an aerobic organism such as was used by ROSSI. Flax straw was put into a large glass vat covered with distilled water and aerated for a week, with frequent examinations for aerobic pectin fermenting organisms.[15] No success was obtained, even after specimens of soil and decaying organic matter were added. Several workers have mentioned the use of aerobic strains. One who is familiar with bacterial metabolism would expect to find an anaerobic organism. Members of this group decompose more materials than aerobes which leave so much energy in their products. The anaerobes partially hydrolyze large amounts of material for a certain amount of energy, while the aerobes hydrolyze a smaller amount of material completely for the same amount of energy. Perhaps for the same reason the anaerobes are used in most of the fermentations which yield certain organic chemicals.

The study of the vat liquors from a large commercial rettery in eastern Michigan, and from the specimens of flax and retting liquors in small tubs in the laboratory, narrowed down to an anaerobic organism as the most specific. This was secured in pure cultures by anaerobic plating in plain agar. Transfers made into other media allowed the following characterization:

[15] Industrial retting of textile plants by microbiological action. Bull. Agric. Intell. 8:1067–1074.

Vegetative cells: The vegetative cells grown on common media were large rods with a dense protoplasm. Many of the cells presented a granular structure. Iodine staining indicated the presence of starchy materials.

Spores.—Spores are formed which are larger than the vegetative cells, giving the cells the shape of a *Clostridium*. The spores were found to resist heating for thirty minutes at 80° C. Further studies on their thermal resistance seemed uncalled for in a study of this nature.

FERMENTATION REACTIONS.—Large amounts of gas were formed in lactose, glucose, saccharose, and glycerol. In most of these fermentation tubes there was a pronounced odor of butyric acid.

LITMUS MILK.—Litmus milk was quickly decomposed; the curd was peptonized with large amounts of organic acids, principally butyric.

GELATIN.—Gelatin was quickly liquefied at 20° C.

PLAIN BROTH.—The broth was rendered cloudy with a precipitate only after a long period of growth.

NITRATES.—Nitrates were reduced with the formation of nitrites and ammonia.

That this anaerobic organism is common in nature and on the stalks of the flax plant was shown in several ways. It was found to be present repeatedly on the stalk of flax by simply soaking it in distilled water. After about thirty hours a vigorous evolution of gas would start, which ceased in about forty-eight hours at room temperature. This could be reproduced at will. That the organism is present in soil was shown by adding garden soil to tubes of sterile flax in distilled water. All evidences of a rapid retting started in twenty-five hours, and was completed in forty-eight hours. Pure cultures of this anaerobe removed the carbohydrate binders in the flax stalk in forty-eight hours at room temperature (26°–32° C.). The fermentation is accompanied by a vigorous evolution of gas, which is forced out of the stalk, clinging to the side until the bubble is large or some jar removes it. The liquid becomes turbid and has a strong characteristic odor.

Whether pure cultures of this anaerobe would be valuable in retting is probably doubtful, since the organism is so widely distributed in nature. Several experiments were carried out to deter-

mine whether the organisms were on the flax stalk itself. There was no difficulty in the majority of attempts to demonstrate its presence. In a large sense the change brought about in the flax stalk is a natural one, which is continually going on in nature. It is an attempt on the part of nature to bring about the transformation of organic compounds, and to keep the elements moving through their cycles. In retting it is the desire to carry this to the point when the binding materials in the fiber are dissolved, thus releasing the bast fibers, and to check it just before the cellulose of the fibers is attacked. It is reasonable to expect that this could be done more quickly in a rettery, where favorable conditions are maintained and where the flora of microorganisms may easily be established.

The quality of the water seems to have great influence on the quality of fiber. During a few experiments in the beginning of this work tap water containing about 1 p.p.m. of iron was used. This yielded a fiber which was dark and discolored in appearance. The use of pure distilled water corrected this and yielded a silken glossy fiber nearly white in color. This supports the experience in water retting that a better fiber is secured where a softer water is available, and confirms the statements of workers that the quality of fiber produced in the Courtrai region in Belgium, where flax is retted in the waters of the river Lys, is superior to that retted elsewhere.

One of the earlier investigators stated that the presence of aerobic bacteria tended to produce more favorable conditions in which the anaerobic could act. To determine whether there was foundation for this, several experiments were carried out with mixtures of the anaerobe isolated in this investigation and certain common aerobes. *Bacillus subtilis* and *Bacterium coli* were used, but it could not be seen that they were of any value. Neither did they seem to lengthen or shorten the time required for completion of the retting process. Their presence seemed to have little effect. Under natural conditions they might favor the retting in that they would help to remove the products formed from the pectic binding materials. In favor of this assumption is the fact that nature does most of her work with mixed cultures, and many significant changes are brought about by symbioses.

Summary and conclusions

1. Retting of flax in the preparation of linen fiber is a natural process, and an attempt on nature's part to keep the elements moving through their cycles.

2. The organism isolated as the specific one in retting flax was *Clostridium amylobacter*. It is an anaerobic spore-forming bacterium which quickly hydrolyzes the carbohydrate "binders" in the flax stalk. It was found to be commonly present on flax stalks and widely distributed in nature.

3. Symbiosis of this organism with common aerobic bacteria did not seem to decrease the time required for retting or produce conditions under which the anaerobic *Clostridium amylobacter* could work better.

4. Temperature is an important factor in that it retards or increases the activity of the fermentation involved in retting. The best temperature seemed to be 30° C.

5. The retting process can be shortened and a better quality of fiber produced by carrying it out under controlled conditions where the optimum environment may be maintained.

6. Previous sterilization of the flax did not seem to affect the retting process. The flax retted as quickly when put into the water without previous treatment as when it was boiled or heated in the autoclave.

7. No real success was secured by the use of fifteen common aerobic bacteria and five yeastlike fungi.

8. Flax raised for seed was quickly retted, although the fiber was not in as good condition as that prepared from flax raised for fiber.

UNIVERSITY OF ILLINOIS
URBANA, ILL.

ANATOMY OF A GALL ON POPULUS TRICHOCARPA

KARL C. HYDE

(WITH PLATE VI)

Early in the fall of 1919 the writer collected some galls upon the twigs of *Populus trichocarpa* T. and G., caused by *Macrophoma tumefaciens* Shear,[1] in Greenough Park, Missoula, Montana. These galls were relatively large woody growths, and appeared on the trees in great numbers. Within an area of about one acre, at the north edge of the park, there were as many as twenty-five trees of various sizes bearing galls, while the trees throughout the rest of the park were practically free from knots. A close examination of the infected trees showed that nearly every twig bore from one to many galls, and that above these galls in many cases the twigs were gradually dying. The disease, although apparently occurring only in isolated localities, appeared to the writer to merit further investigation, and accordingly the problem was attacked at such an angle as to show, if possible, what effect the fungus had upon the normal twig to bring about the hypertrophy resulting in the gall.

Introduction

Populus trichocarpa is the largest tree of the genus, sometimes attaining a height of 65 m. and a diameter of 2.6 m. It is a rapid grower, and is usually found in the lowlands. It is very common throughout the Pacific coast region along the banks of streams, from southern Alaska to northern California, extending as far east as the ontinental divide in Montana. In Washington, Oregon, and eastern Montana it is the largest of the broad-leaved trees, tending to break somewhat the monotony of the vast stretches of coniferous forests of the region in its range. The tree is widely used for shade and ornament for parkings and lawns of the cities of the northwest. In Missoula, for example, there are many trees of this species within the limits of the city. It is especially adaptable for regions of light

[1] HUBERT, E. E., A new *Macrophoma* on galls of *Populus trichocarpa*. Phytopath. 5:182. *figs.* 3. 1915.

rainfall because of its long roots and ease of propagation. The roots were formerly used by the Indians of California and Oregon in the manufacture of hats, baskets, mats, and other ornaments, being well adapted for this purpose because of their toughness, fineness, and length. The wood is dull brown, with nearly white sapwood, soft, light, and weak, with a specific gravity of 0.38. It shrinks moderately, warps considerably, is easily worked, but is not durable. It has a dull silky lustre, and is used extensively for cooperage, boxes, tubs, bowls, canoes, wooden legs, and paper pulp.

The only published work that treats of this gall is that of HUBERT, who deals with the characteristics of the fungus which is thought to be the cause of the gall. He originally found the galls on twigs of *Populus trichocarpa* in 1909, and at first thought they were caused by an insect, *Saperde populnea* L. In March 1910 another collection was made, and he identified the causative organism as a species of *Macrophoma*, but was unable to determine its specific identity. In November 1910 he sent samples of the galls to C. L. SHEAR, United States Bureau of Plant Industry, who described and named the fungus *Macrophoma tumefaciens*.

According to HUBERT, the fungus and galls are widely distributed throughout Montana on the twigs of *Populus trichocarpa*. The several cities mentioned as localities in which he has observed the galls show that they occur at least throughout the western part of Montana and the eastern part of Idaho.

Method

As the xylem of the normal wood is comparatively soft, no difficulty was experienced in obtaining satisfactory sections. Sections of the galls were made with a sliding microtome because they were more uniform in thickness, and good photomicrographs could be secured more readily. First the knots were cut into cubes of suitable size for clamping into the microtome. These cubes were then placed in a mixture of equal parts of glycerine, alcohol (95 per cent), and water, and heated for one hour at a temperature of 100° C. This treatment served the double purpose of softening the wood and of removing a considerable amount of air. All of the air, however, was not eliminated by this process, and it was necessary

to use the airpump to remove entirely the air from the blocks. Sections 10 μ in thickness were easily obtained by these methods. In staining sections it was found that Delafield's haematoxylin in combination with safranin gave satisfactory results.

Anatomy of healthy stem

XYLEM.—The normal wood is of the diffuse, porous type (fig. 1), corresponding in this respect to other species of *Populus*. The vessels are numerous, visible to the naked eye, and are slightly larger in the spring wood than in the summer wood. They form a conspicuous ring of large pores within the earlier wood where it joins with the late wood of the previous season. In most growth rings an irregular, oblique, tangential-radial arrangement of pores can be seen, which, however, do not cross the junction of the spring wood and summer wood of the preceding season's growth. The vessels are of the bordered pitted type in the xylem of secondary growth. In the xylem that arises from the growing point, however, a great many vessels with spirally thickened walls are formed.

MEDULLARY RAY.—The rays of this species, as in all the species of *Populus* which the writer has examined, are scarcely visible with the ordinary hand lens. Only uniseriate rays have been found to occur in normal wood, the cells of which are richly supplied with simple pits (fig. 3). The ray is made up of uniform parenchyma cells elongated horizontally. Viewed radially, a ray appears as a muriform structure composed of several rows of tabular cells. The walls of the cells of the medullary ray are pitted, and the walls therefore have a lattice-like appearance when seen in radial section. The ray cells in most cases contain a considerable amount of starch.

In tangential section, the cross-sections of the rays are shown to good advantage. The uniseriate character is very evident. The rays vary from three to seventeen cells in height, and are distinctly spindle-shaped, the end cells tapering to a decided point. The cells between the end cells vary in shape from cubical to prismatic (fig. 3).

In the radial aspect one is best able to study the wood fibers that compose the bulk of the xylem. These wood fibers are slender, non-septate, spindle-shaped, sharp-pointed cells with narrow cavities. They extend nearly parallel to one another, and diverge from their

course only in weaving around the medullary rays. The wood fibers are perforated with numerous, almost circular bordered pits.

In cross-section the fibers are nearly square in outline, and have relatively thin walls. The walls are somewhat thicker in the summer wood than in the spring wood. The fibers, according to RECORD,[2] have a maximum length of 1.90 mm., a minimum length of 0.50 mm., and an average length of 1.15 mm.

BARK.—The bark as herewith considered includes all that portion of the stem outside the cambium layer, and is composed of epidermis, cortex, and phloem.

The epidermis of young twigs is smooth and colorless. Beneath this is found a layer of parenchyma, from four to seven cells thick, encircling the stem. As the stem becomes older the outer tissues disappear and are replaced by suberized tissue produced through the activity of the phellogen. In the case of normal twigs this cork tissue never becomes very thick. The fibers are thick-walled elements, with sharp pointed, unbranched ends. They are arranged in groups which are in turn arranged in bands that extend around the stem, concentric with the cambium ring.

The phloem is composed of sieve tubes, companion cells, and phloem parenchyma. In many of the cells comparatively large spherical crystals are found.

The pith rays extend into the phloem. They are uniseriate, but broaden considerably as they progress into the cortex. This broadening is due to the fact that the cells are thickened tangentially, and not to any increase in number of rows of cells.

PITH.—The central pith of the stem is composed of cylindrical cells with thin walls. The pith area is about 0.8 mm. in diameter, and varies in color from a light gray to a reddish brown. Many of the cells contain starch and crystals. The crystals are spherical in shape, and never more than one is found within a cell. The outline of the pith area in cross-section is usually distinctly five-angled, but all gradations between this and a circular outline occur.

CAMBIUM.—The cambium of this tree is similar to that of other dicotyledons. It consists of a layer of thin-walled, delicate, tabular

[2] RECORD, S. J., Economic woods of the United States. New York: Wiley and Sons. 1919.

cells. These cells have their long axes extending vertically, and are wider tangentially than radially.

Anatomy of gall

GROSS ANATOMY.—Many galls were examined and were found to vary considerably in size. The largest one measured was 3.5 cm. in diameter, and the smallest one had a diameter of 0.5 cm. They vary in shape from ovoid to globular, and usually encircle the stem. Ordinarily the galls appear singly, but it is not unusual to find them very close together or even confluent on the stem, and characteristically at the nodes. The outer surface of the gall is roughened much more than the outer portion of the normal bark, either above or below the hypertrophy. There has been such a development of suberized tissue on the periphery of the gall, due to the presence of the disease, that it becomes broken into deep, irregular, longitudinal fissures (fig. 5).

Pycnidia of *Macrophoma* are scattered irregularly over the surface of the gall. These pycnidia are flask-shaped, and are imbedded in the parenchymatous tissue of the gall. They have a pseudoparenchymatous wall and open to the exterior through an ostiolum. The pycnidia are barely visible to the naked eye, and are more abundant in the region of the fissures. Considerable sloughing of the bark from the surface occurs, especially at the time of the increased activity of the cambium region in the spring.

It is interesting to note the enormous increase of the hypertrophied part of the stem in comparison with the apparently normal stem above and below the gall. No less interesting is the macroscopic comparison of the normal wood and bark of the region below with the region through the gall (fig. 8). It shows to advantage the relative amount of increase of the tissue in question, as a result of the stimulation of the pathogene. The averages, obtained from the measurements of twenty-five galls of various sizes, are as follows:

Radius of galls	10.5 mm.
Radius of wood of galls	7.0
Thickness of bark of galls	3.5
Radius of stem immediately below gall	5.0
Radius of wood immediately below gall	4.5
Thickness of bark immediately below gall	0.5

It is apparent from these figures that the diameter of the normal stem is composed of 10 per cent bark and 90 per cent wood, while in the diseased twig 25 per cent is bark and only 75 per cent is wood. The section through the gall has increased 110 per cent, the wood 55 per cent, and the bark 600 per cent over the same tissues in the stem just below the gall.

ORIGIN.—The hypertrophy first makes its appearance on the twig in the form of a slight swelling, at or about the time of renewed cambial activity in the spring, which for the region of western Montana is about the first of May. This original swelling is brought about by the increase in number and also the increase in size of the cells of the recent phloem and xylem as secondary growth takes place. It would appear evident, therefore, that this increase in size and number of the cells is brought about by the stimulating effect produced on the cambium by the presence of the fungus. To say that this is due to enzymatic action is only a conjecture, but this is the most plausible explanation.

In the majority of knots sectioned the distortion reached to the pith, at least in some portion of the stem, which indicates that infection took place in the infancy of the twig, as xylem once formed in the region surrounding the pith ordinarily is not subsequently distorted. This, coupled with the fact that the hypertrophy is almost always formed where the twig branches, indicates that the pathogene gains entrance to the host in the region of, and during the formative period of the lateral buds.

The galls evidently arise as the result of the original infection. Frequently when a young lateral twig becomes infected its growth is stunted distad to the infection, and as a result numerous short lateral twigs are present that simulate spurs. Although these spurs may be only a few millimeters in length, they usually show several years growth, as evidenced by the number of terminal bud scars present. These spurs usually protrude from the branch at a distinct right angle, while the normal twig protrudes at an angle of approximately 30° (figs. 5–7). This increased angle is brought about mechanically, the gall forming in the axil exerting a pressure that forces the spur downward.

XYLEM.—In looking at a transverse section of diseased wood such as is shown in fig. 2, one is impressed by the enormous broadening

of the rays. In this view the rays are clearly seen to be multiseriate. Occasionally the rays are seen to join together, giving rise to still wider ones. In a great many cross-sections the rays do not take a direct course through the xylem, but are often broken and their course considerably interrupted. In many cases this ray parenchyma is scattered among the wood fiber and wood parenchyma elements.

The vessels have become greatly distorted throughout (fig. 2). This is due to the flattening of the tubes in a radial direction. In most cross-sections the pores are few in number, and in sections of some galls they are entirely absent (figs. 2, 9). Considerable increase in wood parenchyma cells is seen in the diseased wood. In a great many cases the wood fibers are bent at right angles, the bend always being toward the periphery. Due to the bending, a transverse section often shows these fibers in a longitudinal view (fig. 2). This distortion is brought about largely as a result of crowding, due to the great increase in the number and size of the cells of the medullary rays.

In fig. 4 is shown a tangential section of diseased wood through a gall which illustrates to good advantage the characteristics of the medullary rays, which vary from one to several cells wide tangentially. This broadening is due to the increase in number, as well as to the increase in size of the individual cells making up the rays. That these ray cells are larger in the diseased wood than in the normal wood is apparent by comparison of fig. 3 and fig. 4, the magnification in both cases being the same. It is also evident that there is considerable increase in the size and number of the wood fiber and wood parenchyma elements. The medullary rays sometimes become so broad that their tangential diameter equals, or is even greater than their vertical diameter (fig. 4).

The average of one hundred measurements of the diameter of the medullary ray cells, in the tangential sections of normal and diseased wood of the same age, gave for the former 13.2 μ and for the latter 27.9 μ. This shows an increase of slightly over 100 per cent in these cells as a result of the diseased condition. Measurements of the diameters of the wood fibers in the same sections gave an average in the normal wood of 12 μ and in the diseased wood an average of 16 μ.

Here and there in the xylem of the diseased area uniseriate rays occur, but these are far outnumbered by the multiseriate rays. The ray tissue in the diseased xylem makes up approximately 30 per cent of the wood, while it is evident, by comparing the figures, that in the normal wood the ray tissue makes up a much smaller proportion.

BARK.—The distortion of the elements of the bark of the gall are not so pronounced as in the xylem. In the bark the principal effect is found to be a decided increase in size and number of the cells of the several tissues. As a result of the normal reaction of the host, in an attempt to overcome the injurious effects of the pathogene, the amount of suberized tissue is increased manyfold, so that now there are as many as fifty rows of cork cells. The parenchyma cells of the primary cortex are considerably larger and more than doubled in number. The average of numerous counts made of the number of rows of parenchyma cells between the cambium and the periderm in normal and diseased bark shows for the former 28, and for the latter 65. The phloem rays are multiseriate, often comprising six or seven rows of cells. These rays are sometimes bent tangentially, as seen in cross-section.

The phloem tissue is greatly increased in the diseased twig. This increase is largely due to the multiplication of the phloem parenchyma cells and the subsequent growth of the cells to a size slightly beyond the normal. Physiologically the phloem does not appear to be greatly interfered with during the younger stages of the gall. As the gall becomes larger, considerable pressure is exerted upon the sieve tubes, as is indicated by the fact that they are flattened radially.

The functional disturbance seems to be more closely connected with the xylem than with the phloem, as it is here that the distortion of elements is the greatest. This disturbance is manifested in the gradual dying of the twig above the hypertrophy (fig. 7), which, however, does not usually occur unless there are several knots upon the twig. In cases where only one knot is found on the twig there usually is no noticeable disturbance distad to the knot.

The writer believes the death of the twig to be due to the fact that sufficient water and minerals cannot get through the vessels of the xylem to the leaves beyond the galls. The supply of water and

minerals is shut off because the xylem elements are so twisted and distorted that the vessels as vertical tubes and efficient water carriers have largely disappeared. The water and mineral nutrients are able to pass slowly one or two galls by diffusion, even though the vessels are greatly distorted, but when several galls are present in the path the movement is so interfered with that growth is retarded and sooner or later the tip part of the twig dies above the knot.

PITH.—No striking effects on the central pith as a result of the gall formation are noticeable, but in some larger galls the pith area is somewhat compressed, and the individual pith cells in these cases have lost their characteristic cylindrical form and have taken on an angular appearance.

CAMBIUM.—The cambium appears to be very active in the younger galls, as evidenced by the comparatively larger cells and nuclei. As the gall grows older this activity gradually grows less, until it is brought to an end by the death of the twig. In cases where death of the twig distad to the gall does not occur, the activity of the cambium eventually becomes almost negligible. The cambium ring as a whole becomes greatly distorted and interrupted in many places. The wood fibers that are bent toward the periphery are responsible for this interruption of the cambium. The cambium never entirely loses its identity (fig. 9). Many nascent cells are isolated from the cambium that give rise by continual division to isolated groups of phloem in the xylem region. These groups are more often formed between the spring wood and the summer wood of the preceding years' growth. They are usually crescent-shaped, and suggest the pith flecks often found in other woods. There are no isolated xylem groups formed in the phloem, but a great many wedgelike xylem elements extend through the cambium into this region (fig. 9).

Summary

1. The normal wood of *Populus trichocarpa* conforms closely to a typical dicotyledonous, diffuse, porous wood.

2. In western Montana and eastern Idaho a gall disease threatens to interfere with the commercial uses of this tree.

3. Only uniseriate rays are found in the normal wood of this tree, while in the diseased wood the rays are considerably broadened, often being several cells wide tangentially.

4. The average increase in the diameter of the stem, due to gall formation of several galls measured is 110 per cent, of xylem 55 per cent, and of phloem 600 per cent.

5. The xylem elements are greatly distorted, the vessels are flattened radially, and the wood fibers are often bent at right angles, due to crowding as a result of the great increase in number and size of the cells of the medullary ray.

6. In the bark the greatest effect noticeable is the increase in size and number of the parenchyma cells.

7. The phellogen is stimulated to unusual activity, and consequently the suberized tissue is considerably increased.

8. The distortion of the vascular elements, because of the interference with the transpiration stream, often results in the twigs dying above the galls.

9. The central pith is not greatly altered in the diseased stem.

10. The cambium is sometimes altered by distortion, but never completely loses its identity.

11. In addition to the evidence of constant association of *Macrophoma tumefaciens* with the lesions, the histological examination supports the idea that this fungus is the cause of the disease.

12. Infection experiments on pathogenicity are as yet lacking.

Acknowledgment is made to Professor W. W. ROWLEE, under whose direction this study was made, and to Professor D. REDDICK for many suggestions in the preparation of the manuscript.

CORNELL UNIVERSITY

EXPLANATION OF PLATE VI

All photomicrographs show magnification of 85 diameters.

FIG. 1.—Transverse section of normal wood, showing one annual ring complete.

FIG. 2.—Transverse section of diseased wood, showing distorted multiseriate medullary ray and radially flattened vessels.

FIG. 3.—Tangential section of normal wood, showing uniseriate medullary rays and normal fibers.

FIG. 4.—Tangential section of diseased wood, showing broadened medullary rays and enlarged and distorted fibers.

FIG. 5.—Twigs bearing characteristic galls; reduced $\frac{1}{3}$.

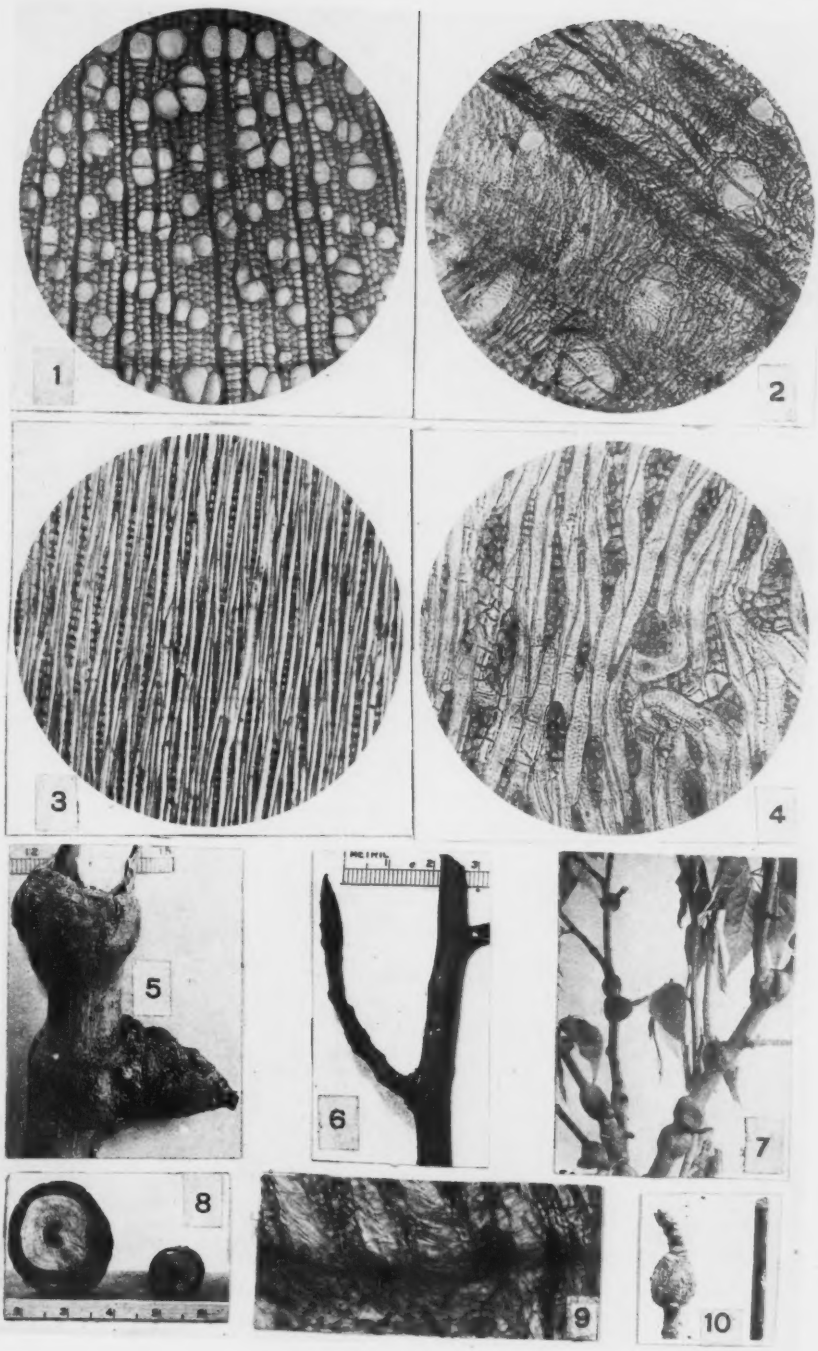
FIG. 6.—Normal twig, showing method of branching; reduced $\frac{1}{3}$.

FIG. 7.—Cluster of branches, showing characteristic habit of galls; many of these twigs were dead at tips; much reduced.

FIG. 8.—Transverse section of gall on five-year old twig; to right of this is shown transverse section of twig just below gall; reduced $\frac{1}{3}$.

FIG. 9.—Characteristic irregularity of cambium ring in diseased twig.

FIG. 10.—Normal one-year old twig and twig of same age bearing gall; reduced $\frac{1}{3}$.



HYDE on POPULUS GALL

POLLINATION IN ALFALFA

F. A. COFFMAN

(WITH FIVE FIGURES)

The problem of fertilization in alfalfa has been a matter of considerable controversy for several years. Many experiments have been performed in endeavoring to determine what factor is most largely responsible for pollination in this plant. In the review of investigations published by PIPER, EVANS, MCKEE, and MORSE[1] it is stated that the amount of self-pollination varies with the season, and that its real importance in seed production is doubtful. HILDEBRAND[2] is cited as having believed as early as 1866 that fertilization may take place in untripped flowers, and URBAN[3] (1873) is referred to as thinking that in some cases untripped flowers form pods. Most of the work of recent investigators has consisted of attempts to determine the most important agents in tripping the alfalfa blossom. Although a considerable number of experiments have been carried on, no definite conclusions seem to have been reached. ROBERTS[4] found anthers dehiscing and stigmas pollinated in the early bud stages of the alfalfa.

An investigation which was begun by the writer during the latter part of September 1916, and which was continued until killing frosts in October, had for its object the determination of the stage at which the stamens of the majority of alfalfa flowers really shed their pollen. It was found that light frosts do not seem to have any effect upon pollination, the percentage of pollinated to unpollinated flowers not being affected so far as could be noticed in any of the classes of buds examined.

[1] PIPER, C. V., EVANS, M. W., MCKEE, R., and MORSE, W. J., Alfalfa seed production; pollination studies. Bull. 75. U.S. Dept. Agric. 1914.

[2] HILDEBRAND, F., Über die Vorrichtungen an einigen Bluthen zur Befruchtung durch Insektenhulfe. Bot. Zeit. **24**:75. 1866.

[3] URBAN, I., Prodromus einer Monographie der Gattung *Medicago*. Verhandl. Bot. Ver. Provinz Brandenburg **15**:13. 1873.

[4] ROBERTS, H. F., Alfalfa varieties, breeding, seed, and inoculation. Quarterly Rept. Kans. State Board Agric. **35**:180. 1916.

The flowers were divided arbitrarily into four classes, according to the stages of their development, as follows: straight bud, fig. 1; pointed bud, fig. 2; hooded bud, fig. 3; and erect standard, fig. 4. The lengths of the flower in millimeters were determined before they were examined, and these measurements serve to some extent as checks on their stages of development. In the fourth class of blossoms, the flowers did not measure longer than in the hooded bud

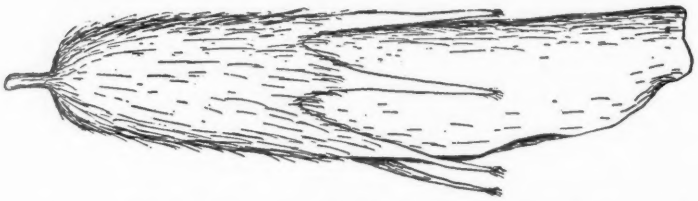


FIG. 1.—Alfalfa bud representing "straight bud" stage; corolla in this stage has not grown out much beyond calyx; only standard petal can be seen; it is folded around all of the others.

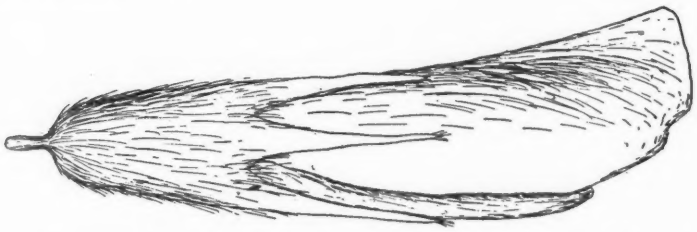


FIG. 2.—Alfalfa bud representing "pointed bud" stage; standard petal is seen to be distinctly curving upward in its growth; lower edges of wing petals, which together fold down over keel, are visible.

stage. This is because all measurements were taken from the base of the calyx to the tip of the flower in the first stages. After the standard began to arise, the measurements continued to be taken to the tip of the interlocking envelope of the wings and keel, which did not further elongate after the erection of the standard. After being measured, each flower was dissected by means of needles and forceps at a magnification of 23 diameters, under a Zeiss binocular microscope, using F-55 objectives and no. 5 oculars. The dissec-

tions were made by removing first the standard, and then one of the wing petals, thus exposing the interior of the flower to view. Care was taken not to set off the tripping mechanism, nor to disturb

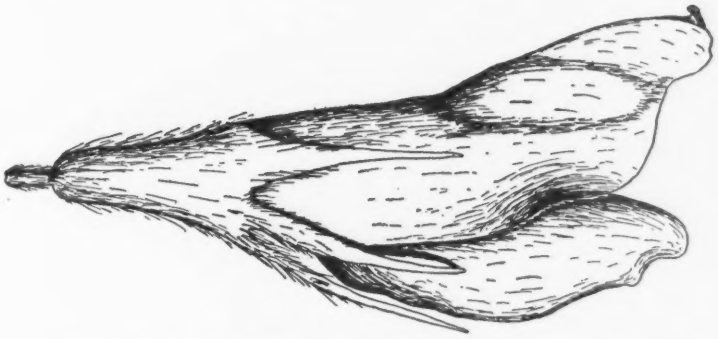


FIG. 3.—Young alfalfa flower called "hooded bud" stage; standard petal has risen nearly to full height, and is beginning to spread; wing petals distinctly seen protruding, folded over keel, which is not yet visible.

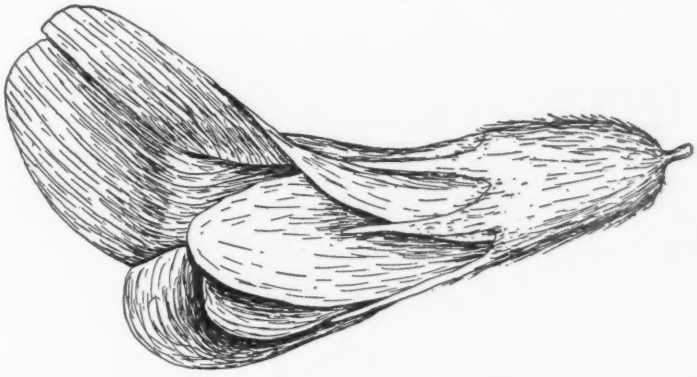


FIG. 4.—Alfalfa flower fully open and ready for tripping, representing "erect standard" stage; standard fully risen and spread; wing petals are separating, showing keel within.

the stamens or otherwise injure the flowers. Flowers believed to have been injured in the dissecting process were discarded and not recorded.

The ratio of buds with anthers dehisced to those with anthers intact, in the pointed bud stage, was surprisingly close. Probably twenty flowers of the straight bud stage, in which the anthers had dehisced, were discarded, since it was thought that they might have

TABLE I

Flower length (mm.)	Anthers intact	Anthers dehisced	Stigmas pollinated
	Straight bud stage		
5	20	1	1
6	49	1	1
7	12	1	1
8	.....	1	1
Total	81	4	4
	Pointed bud stage		
6	18	1	1
7	44	22	19
8	21	41	37
9	3	19	19
10	.....	1	1
Total	86	84	77
	Hooded bud stage		
7	.....	1	1
8	.....	8	8
9	2	18	18
10	1	9	9
Total	3	36	36
	Erect standard stage		
7	.....	1	1
8	.....	2	2
9	.....	13	13
10	.....	4	4
11	.....	1	1
12	.....	1	1
Total	.....	22	22

been injured by dissection, thereby causing the bursting of the anthers. In handling the flowers of the hooded and erect standard stages, care was exercised to prevent them from tripping. The standard was first removed so as to keep the anthers from striking

it and from being broken, should the flower accidentally be tripped. One of the wing petals was then pulled away and the stamens examined. Most of the flowers in the erect standard stage, as they were found in the field, had already been tripped, untripped flowers being rather difficult to secure. A total of 316 flowers was examined and recorded as follows:

Straight bud stage.....	85	Hooded bud stage.....	39
Pointed bud stage.....	170	Erect standard stage.....	22

In the straight bud class, the flowers measure 5–8 mm. in length, and of the total number recorded, in only four instances had the stamens shed their pollen. The flowers in the pointed bud stage are considerably longer, being 6–10 mm. in length, and practically one-half of those recorded were pollinated. The hooded and erect classes of buds do not differ greatly from each other in size, varying from 7 to 12 mm. in length. But three flowers in the hooded bud stage had not been pollinated, while no unpollinated erect standard blossoms were found. The data as taken in this investigation, on the stages of bud development in relation to pollination, are given in table I.

From the data secured, the percentages of pollination of the flowers of different lengths, regardless of their stages of development, are given in table II.

TABLE II

Flower length (mm.)	No. examined	No. with anthers intact	Percentage with anthers intact	No. with anthers dehisced	Percentage with anthers dehisced
5.....	21	20	95.24	1	4.76
6.....	69	67	97.11	2	2.89
7.....	81	56	69.14	25	30.86
8.....	73	21	28.77	52	71.23
9.....	55	5	9.09	50	90.91
10.....	15	1	6.67	14	93.33
11.....	1	0	0	1	100.00
12.....	1	0	0	1	100.00
Total....	316	170	.....	146	.....

According to table II, anthers do not seem to shed their pollen before the flowers have reached 7 mm. in length, and most of them have done so by the time they are between 9 and 10 mm. long.

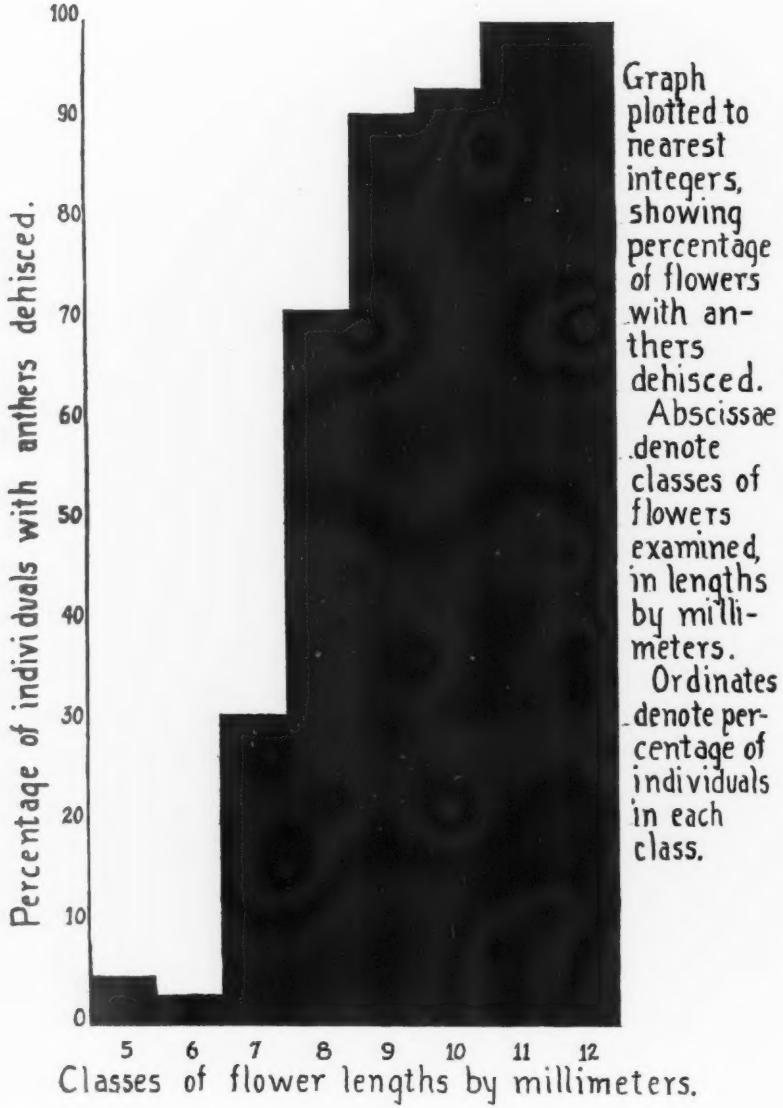

100
90
80
70
60
50
40
30
20
10
0
Percentage of individuals with anthers dehisced.
5 6 7 8 9 10 11 12
Classes of flower lengths by millimeters.
Graph plotted to nearest integers, showing percentage of flowers with anthers dehisced.
Abscissae denote classes of flowers examined, in lengths by millimeters.
Ordinates denote percentage of individuals in each class.

FIG. 5

This relation between dehisced anthers and flower length is shown graphically in fig. 5.

From the data secured as the result of this preliminary investigation, it appears that practically all alfalfa blossoms shed their pollen during the pointed bud stage, and before the hooded bud stage is reached. Judging by this, it would seem that tripping is not essential to pollination.

The alfalfa flower apparently begins to shed its pollen while yet comparatively small, being only about 7 mm. in length, which in hybridization operations would necessitate emasculation being accomplished while the blossom is still in the straight bud stage, in order to eliminate all possible danger of self-fertilization. As a matter of fact, this is a practical impossibility from the mechanical standpoint in the field at least, the flowers being emasculated in alfalfa crossing work by tripping them not earlier than the hooded bud stage and generally later, the pollen then being washed out with an atomizer spray. It is impossible to emasculate the flowers earlier than the hooded bud stage. The writer believes, however, that there is great danger of pollination of the stigma and of consequent self-fertilization, before the time when the flowers are ordinarily tripped in alfalfa by hybridizing operations.

This investigation was carried on in connection with graduate work under the direction of Professor H. F. ROBERTS, of the Department of Botany, Kansas State Agricultural College. The illustrations are from pp. 202–203 of his paper referred to herein.

U.S. DEPARTMENT OF AGRICULTURE
AKRON, COLO.

PROTECTIVE POWER AGAINST SALT INJURY OF LARGE ROOT SYSTEMS OF WHEAT SEEDLINGS

W. F. GERICKE

That wheat plants can be made to grow very large root systems as compared with the growth of their tops by certain properties of nutrient solutions, has been shown in a previous paper.[1] The writer has also shown that under certain conditions large root systems of wheat seedlings 4–6 weeks old play an important rôle in the number of tillers[2] the plant may produce. These observations suggested further experimentation where differences in the extent of the root systems of the plants would enter as the variable factor. It seemed plausible to expect that the relative physiological values or growth efficiencies of different nutrient solutions, and the tolerance of plants to salts, were not inconsiderably affected by the extent of the root development of the test plants when placed in the media. The present paper bears upon an investigation on these points.

Three different kinds of nutrient solutions were selected for the tests. These were solutions whose relative values as growth media had previously been obtained. The composition, molecular concentration of the salts, and the relative physiological values of these solutions stated as "good," "medium," or "poor" were as follows:

Solution no. 1.—0.0102 mol. KH_2PO_4; 0.0057 mol. $Ca(NO_3)_2$; 0.0062 mol. $MgSO_4$. Good.

Solution no. 2.—0.014 mol. K_2SO_4; 0.002 mol. $Ca(NO_3)_2$; 0.002 mol. $Mg(H_2PO_4)_2$. Very poor.

Solution no. 3.—0.016 mol. $MgSO_4$; 0.002 mol. $Ca(NO_3)_2$; 0.002 mol. KH_2PO_4. Poor if air temperature and transpiration for growth were high; medium if air temperature and transpiration for growth were relatively low.

[1] GERICKE, W. F., Root development of wheat seedlings. BOT. GAZ. 72:404–406. 1921.

[2] ———, Certain relations between root development and tillering in wheat. (To appear in Amer. Jour. Bot. 9:1922.)

Sets of eight containers (Mason jars) of one-half gallon capacity were used for each of the different solutions and for each of the two different classes of wheat seedlings. These classes of seedlings were distinguished by a difference in the extent of root growth from that of top growth which the plants had when placed in the nutrient solutions. The method employed to obtain seedlings with large root systems was that referred to in the earlier paper. This consisted in allowing the cultures to grow in one quart Mason jars filled with tap water for five weeks before the cultures were placed in the nutrient solutions to be tested. The plants at this time had a root mass 70–80 cm. long, and had about one-half of their total dry matter in the roots. They were transferred from the tap water directly to the three nutrient solutions to be tested. To grow contemporaneously with these, other seedlings (young plants just germinated and therefore having small root systems) were set out in other sets of containers filled with the nutrient solutions to be tested. The seedlings in this latter case were 6–8 cm. high, with roots 8–10 cm. long, about 20 per cent of the dry weight of the plant thus being roots. Subsequent treatment of all cultures was alike, and this included additions of a small amount of $FeSO_4$ to each culture at regular intervals, also regular additions of distilled water to make up the loss of water by transpiration. The test period was six weeks. The experiment was carried on in the greenhouse during parts of July and August, the range of temperature being 20°–32° C. The relative humidity of the greenhouse did not permit excessive transpiration. At the end of the test period, the plants were harvested, dried, and weighed. Table I gives the data obtained.

In taking up the data in detail, it may be noted that the cultures which had large and extensive roots (class *A*), when placed in the "good" nutrient solution no. 1, produced less than one-half as much total dry matter as did the cultures which were started with comparatively small roots (class *B*). The latter class of cultures at the end of the test period had the largest root growth, exceeding by more than 76 per cent that of the next largest. The cultures of class *B* are to be considered as normal plants when set out, the other class not. Even though class *A* had by far the larger roots when the test was started, it is obvious that these large roots did not operate

as a means to secure as great a rate of growth for the plants, especially for the aerial portion, as was obtained by the plants started with relatively small roots having less surface exposed for absorption

TABLE I

EFFECT ON DRY MATTER PRODUCTION OF DIFFERENT ROOT SYSTEMS OF WHEAT SEEDLINGS GROWN IN DIFFERENT NUTRIENT SOLUTIONS (WEIGHT IN GM.)

	Class A Cultures having large root systems when placed in solution			Class B Cultures having small root systems when placed in solution		
	Tops	Roots	Total	Tops	Roots	Total
			Solution 1			
	1.22	0.42	1.64	3.00	0.73	3.73
	1.13	0.37	1.50	3.29	1.09	4.38
	1.35	0.40	1.75	2.60	0.53	3.13
	1.06	0.38	1.44	3.12	0.95	4.07
	1.38	0.48	1.86	2.95	0.70	3.65
	1.07	0.35	1.42	3.04	0.67	3.71
	1.41	0.55	1.96	3.02	0.62	3.64
	1.15	0.40	1.55	2.97	0.60	3.57
Average	1.26	0.42	1.64	3.00	0.74	3.74
			Solution 2			
	1.65	0.43	2.08	0.62	0.12	0.84
	1.42	0.35	1.77	0.60	0.10	0.70
	1.00	0.36	1.36	0.45	0.10	0.55
	1.49	0.37	1.86	0.61	0.12	0.73
	1.38	0.36	1.74	0.75	0.11	0.86
	1.10	0.39	1.49	0.55	0.09	0.64
	1.32	0.37	1.69	0.53	0.10	0.63
	1.10	0.39	1.49			
Average	1.30	0.38	1.68	0.59	0.11	0.70
			Solution 3			
	1.00	0.39	1.39	2.94	0.35	3.29
	0.98	0.35	1.33	2.96	0.35	3.31
	1.24	0.42	1.66	3.00	0.46	3.46
	1.61	0.48	2.09	2.40	0.27	2.67
	1.00	0.33	1.33	2.97	0.42	3.39
	1.07	0.30	1.37	*1.62	0.21	1.83
	1.05	0.30	1.35	2.50	0.32	2.82
	1.75	0.37	2.12	2.74	0.39	3.13
Average	1.21	0.37	1.58	2.64	0.35	2.99

* Two plants in this culture died.

processes. On the other hand, it is not evident that the large root systems prevented the cultures of class *A* from attaining to the

measure of growth obtained by the cultures of class *B*. That the cultures of class *A* failed to give the measure of growth obtained by the cultures of class *B* must undoubtedly be attributed to some effects of the previous treatment, and of which the large roots in this case apparently may be considered but an incident. In this connection it can be argued that the cultures of class *A* were stunted and did not possess the same potential power or capacity for growth as did those of class *B*, and therefore regardless of any possible beneficial effect, if these large roots meant a greater surface for absorption, this could not compensate to overcome the stunted effects suffered by the plants. Undoubtedly the capacity of a plant to grow is affected by the rate of absorption of nutrients, and vice versa, the rate of absorption of nutrients is affected by the growth of the plants, so that the absorbing capacity of any comparable unit area of root surface must vary with conditions. It appears, therefore, that the data of the cultures grown in solution no. 1, taken by themselves, do not give any indication as to what effects the different root systems had in the results.

The results obtained from solution no. 2 are decidedly different. The seedlings having large roots, when placed in this "very poor" nutrient solution, produced about two and one-half times as much dry matter as did the other class of seedlings grown in this solution. The yield of the cultures of class *A* grown in solution no. 2 were approximately of the same magnitude as those grown in solution no. 1. The yield of the cultures of class *B* having comparatively small roots when placed in solution no. 1 was about five and one-half times larger than that of the corresponding cultures grown in solution no. 2. The explanation for the differences in growth obtained from the two classes of seedlings grown in solution no. 2 seems to be due to differences in the extent of the root systems these cultures had when placed in the media. It is quite obvious that the great difference in total dry weight obtained from the two classes of cultures grown in solution no. 2 is due to the very small growth made by the cultures with small roots, and not to any exceptionally good growth made by the cultures with large roots. The effect of solution no. 2 upon the one class of seedlings (class *B*) was to prevent its making such a measure of root growth as could be necessary to enable the plants to make even a moderate measure of top growth.

The injury to these seedlings was relatively great, therefore, but this was not the case with seedlings having large root growth.

It appears that there are several reasons that can be offered as an explanation for this relatively good growth obtained from the cultures with large roots grown in solution no. 2. The roots of these cultures, presumably because of their age, had much suberized tissue. This could inhibit the entry of excessive amounts of salts. It could also cause the precipitation of some of the salts in the root mass without doing injury to the plants, and, also in a selective way prevent or retard the absorption of toxic ions. On the other hand, this large root system was beneficial to the plant growth in this poor solution, in that it still permitted sufficient absorption of the essential ions. The greater surface exposed to absorption of nutrients, therefore, could compensate for the decrease in the rate of intake of essential nutrients per unit area of root surface.

Results obtained from solution no. 3 show that the plants having comparatively small root systems, when placed in the solution, produced approximately 90 per cent more dry weight than did the other class of cultures grown in this same kind of solution. The dry weight of the cultures of class *B* was also more than four times larger than that of the corresponding cultures of solution no. 2. The growth obtained from class *B* cultures in this solution (no. 3), in which $MgSO_4$ composed eight-tenths of the total salt concentration, must be considered as very good. Had growth conditions prevailed that would have induced a higher rate of transpiration, these cultures would not have attained to the value they held in this test. It is to be noted that these cultures had the lowest percentage of dry matter in the roots of all sets, being 11.7 per cent of the total growth obtained, and constituting a very low value for wheat plants six weeks old. One effect of this solution was to retard root growth in the cultures of class *B* as compared with the growth of top. Under conditions of excessive transpiration this condition would have acted harmfully to the plants. As it was, the cultures of class *B* having the smallest root systems as compared with the tops of the plants appeared to have been the most efficient, if ratio of root growth to top be taken as the criterion.

The dry weight of all the cultures having large root systems (class *A*) when placed in the three different kinds of solutions were

of approximately equal value, having increased approximately five times in weight during the six weeks' period of growth. This test shows that these three solutions, markedly different in composition, must be considered as of equal value as media for the growth of wheat seedlings five weeks old having large root systems, when placed in the solutions and grown for six weeks. These same solutions, however, must also be considered markedly different physiologically, when the test plants are wheat seedlings 6–8 cm. high with small roots 8–10 cm. long.

All the cultures with large roots may be considered as having been injured by the treatment of the first five weeks' growth in tap water, because they fell short in attaining the maximum growth rate obtained by the cultures with small roots (class *B*) in solution no. 1. Whether any treatment that can induce large root growth of a wheat plant, either at the expense of top growth or not, can subsequently be made to operate as a means to secure a greater growth rate for the plant as a whole, because of a greater root surface exposed for absorption, needs further investigation. It is probable that in the present experiment the treatment to obtain large roots was too severe, and that exposure for a less time to the conditions by which large roots were obtained would have given a larger measure of growth when set into these solutions than was obtained.

The extent of the root system appears as an important factor that affects the magnitude of growth obtainable from a given nutrient solution. It is conceivable, therefore, that extent of the root systems of plants plays an important rôle when plants are grown in the field. That some plants are more resistant than others to certain untoward conditions, such as excessive amounts of salts in alkali regions, may not be due to any peculiar genetic factor of the plant, but simply be the response from differences in root development occasioned by certain conditions in the environment in the field. The common observance in the field of a greater tolerance for salts of older plants than young ones apparently can well be accounted for in their root systems. This, however, does not mean that differences in extent of root systems any given kind of plant may have are due only to causes operative in the external environment. Differences in root systems may also be due to genetic factors.

Agricultural Experiment Station
University of California

A NEW FRUIT ROT OF TOMATOES[1]

R. FRANK POOLE

(WITH PLATE VII)

During the summer of 1921 a fungus growth following cracking of the fruit was noted on tomatoes in several localities of Burlington and other counties in New Jersey (fig. 1). The cracking was obvious on both green and ripe fruit of the Stone, Baltimore, and Bonny Best varieties, but was especially prominent and severe on the latter. The cracking is apparently due to one or more physiological causes. An examination showed a very dense fluffy growth of *Oidium lactis* Fres. in the open cracks of ripe fruit lying on the ground and those hanging on the plant to a height of several inches above the soil. This fungus, under field conditions, penetrated the interior of the tomato, and reduced the fruit to a soft rotten mass in from two to five days. The disease was very common throughout the tomato ripening period in the fields under observation. No infection was noted on uninjured ripe fruit, cracked green fruit, or other parts of the plant.

CAUSE.—The rot is due to *Oidium* or *Oospora lactis*. The fungus is repeatedly isolated from infected tomatoes. It causes rapid decay of ripe fruit at 20° C. in a moist chamber. The mold is grayish white, fluffy, and dense (fig. 2). The mycelial growth is more important than spore production. If, however, a diseased tomato be broken open and spread out in a moist chamber for twenty-four hours at 20° C. (fig. 3), the fungus appears very similar in growth to the *Saccharomyces*. In this form the spore production is abundant, while the mycelial growth is subdued. These two factors of mycelium and spore production may be considered as distinguishable characteristics of this fungus from other fruit rot organisms. The fungus grows abundantly on a large variety of culture media. Its only known method of reproduction is non-

[1] Paper no. 60 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Plant Pathology.

sexual, typically by means of conidial spore chains. The spores are hyaline, round or oval, and smooth. They vary in size from 2 to 6 $\mu \times 6$ to 40 μ (fig. 5).

SYMPTOMS.—The fungus causes a typical soft rot of injured, ripe tomato fruits. In some cases there is a fermentation action, due to the fungus, which causes the cracks to widen and the juice to flow out (fig. 2). The inner tissue is destroyed, while the peeling is not noticeably attacked, but dries out and remains on the field as a dry, hard shell. In advanced decay the symptoms are not easily distinguished from those of a bacterial soft rot of ripe tomato fruit. The odor is at first agreeable, but becomes very offensive before decay is complete. Decay of cracked green fruit was not observed in the field, but the fungus caused slow decay (fig. 4) in fruit that had begun to ripen.

INOCULATIONS.—Ripe, semiripe, and green tomatoes were placed in running water for an hour, treated fifteen minutes with bichloride of mercury, washed with sterilized water, and placed in dry sterilized glass chambers prepared for inoculation. No infection was obtained by spraying spores on uninjured fruit. The fungus caused rapid decay of sliced ripe tomatoes in four to six days at room temperature, 18–20° C. (fig. 4*a*). It grows very slowly on green or semiripe fruit (fig. 4*b*).

Spores of *Oidium* or *Oospora lactis* were introduced into the solid ripe tomatoes by means of a platinum needle. In forty-eight hours at room temperature there was good growth in all places where inoculated (fig. 2*b*). The growth was abundant for a similar period on deep slices made in the tomato (fig. 2*a*). Ripe tomatoes which were punctured but not inoculated did not become infected in the same chambers where other tomatoes were inoculated (fig. 2*c*).

DISTRIBUTION, PREVALENCE, LOSS.—While no definite data are available to show accurately the distribution of the fungus, THOM[2] states: "The mold variously known as *Oidium* or *Oospora lactis* is another cosmopolitan organism. The same or almost indistinguishable forms are found upon decaying vegetables and fruits, which may give reason for the statement that the odor produced of

[2] THOM, CHAS., Fungi on cheese ripening Camembert and Roquefort. Bur. Animal Ind. Bull. 82. 1–40. *figs.* 3. 1906.

Oidium is that of rotten cabbage." There is considerable literature, too extensive to mention here, dealing with *Oidium lactis* in milk products, particularly the relation of the mold to the flavor of Camembert cheese. Very little of this literature deals with the fungus in relation to plant products. PEROTTI and CRISTOFALETTI,[3] however, have briefly reported the fungus as a parasite appearing in spots on tomatoes in Italy. They suggested that the fungus be called *Oidium lactis solani*.

The largest losses were noted near Moorestown, Burlington County. The disease was prevalent in other localities, and losses were more or less regular over the entire tomato growing area in localities where the disease was observed. While the loss was not serious at any one period, there was a rather high loss for the season.

TABLE I

BONNY BEST TOMATOES

SPRAY TREATMENTS	AUGUST 12			SEPTEMBER 7		
	Examined	Diseased	Percentage diseased	Examined	Diseased	Percentage diseased
1. 4–4–50	171	21	12.2	140	25	17.8
2. Check	95	17	17.7	64	13	20.3
3. 4–4–50	102	12	11.7	120	21	17.5
4. Check	120	19	15.8	65	17	26.1

Data were collected in tomato fields of the so-called second early Bonny Best tomatoes, August 12 and September 7, and from a late crop of Baltimores, September 19. This was during the maximum ripening period of each crop. The data were obtained by counting the total large fruits on ten average plants and also the number that were diseased. It will be noted in table I that the disease was slightly higher on September 7 in the Bonny Best tomatoes than it was on August 12. It is very probable that the conditions favoring infection were more prevalent September 7 than August 12.

The percentages of disease in table II were taken from four series of 4–7 applications inclusive of four liquid spray and dust

[3] PEROTTI, R., and CRISTOFOLETTI, U., A fruit spot of tomato. Staz. Sper. Agric. Ital. **47**:169–216. *pls. 3. figs. 9.* 1914.

treatments. It appears from the data given in this table that the disease was checked with wet Bordeaux and dust treatments. Such an interpretation is no doubt correct, but the difference of control on treated and untreated plots is not entirely due to treatments. There were slightly more diseased tomatoes on the untreated plots than were formed on the treated plots. The total yield was less on the untreated plots, because much of the fruit had prematurely ripened and was picked or had fallen. The calculation of the percentages of the disease on the untreated plots with a smaller total of fruit on the ten check plants than was obtained from ten plants

TABLE II

Wet spray and dust treatments	Series 1		Series 2		Series 3		Series 4	
	No. examined on 10 plants	Seven applications, percentage diseased Sept. 19	No. examined on 10 plants	Six applications, Percentage diseased, Sept. 19	No. examined on 10 plants	Five applications, percentage diseased Sept. 19	No. examined on 10 plants	Four applications, percentage diseased Sept. 19
1. Check.....	166	4.2	110	11.8	123	10.5	130	2.3
2. ½–0–3–50....	192	1.5	142	2.8	116	9.0	168	2.4
3. 4–4–50.....	211	2.9	124	0.8	193	2.0	184	3.2
4. Dust.....	211	1.9	128	1.5	193	2.0	236	.88
5. 4–3–1½–50...	193	1.5	140	0.7	134	4.5	211	.95
6. Check.....	154	3.6	90	8.8	159	6.3	180	3.3
7. ½–0–3–50....	157	2.5	83	4.8	164	3.6	140	2.8
8. 4–4–50.....	142	1.4	126	2.4	209	0	175	1.7
9. Dust.....	169	1.2	138	1.5	154	1.3	185	2.1
10. 4–3–1½–50...	146	0.7	129	0.8	198	9.6	200	0.5

on the treated plots, therefore, has resulted in a slightly increased percentage of disease in the check over the true percentage of control. It will be noted in table II, however, that there is also a difference of control in the four treatments, which indicates that some slight true control was obtained.

The treatments were planned in connection with the investigation of *Septoria lycopersici* control. The third number in the wet Bordeaux spray represents fish oil soap. The dust was composed of 16 pounds anhydrous copper sulphate, 6 pounds lead arsenate, and 78 pounds of hydrated lime. The wet treatments were applied with a 3-row, 3-nozzle traction spray machine, while the dusts were applied with a hand duster.

Summary

1. A cracking of green and ripe fruit of Bonny Best, Baltimore, and Stone tomatoes in Burlington and other counties of New Jersey, due to one or more physiological causes, was observed to be severe in 1921.

2. *Oidium* or *Oospora lactis* was isolated from infected tomatoes. Inoculations of ripe fruit with this fungus were positive.

3. *Oidium* or *Oospora lactis* is a widely distributed fungus. It is known to appear in milk products, cheeses, decaying vegetables, and fruits. On tomato fruit the fungus mycelium is dense, grayish white, and prominent, while in other cases spores are very prominently produced.

4. The treatments with wet Bordeaux sprays and dusts gave slight control of the disease.

Appreciation is expressed for the helpful suggestions offered by Dr. MEL. T. COOK, and for the identification of the organism by Miss ANNA E. JENKINS, Office Pathological Collections, Washington, D.C.

AGRICULTURAL EXPERIMENT STATION
NEW BRUNSWICK, N.J.

EXPLANATION OF PLATE VII

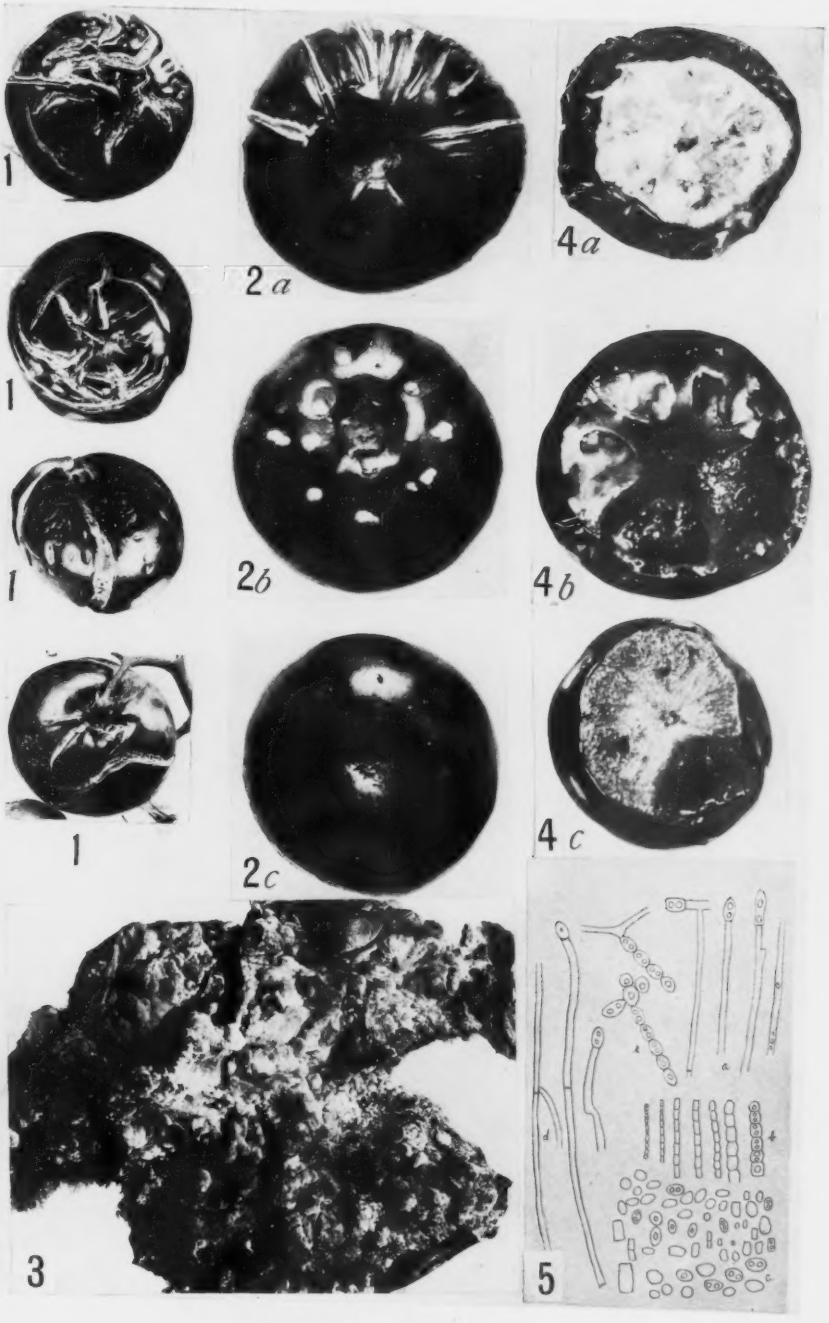
FIG. 1.—Cracked ripe and green Bonny Best tomatoes, showing various forms of cracking.

FIG. 2.—Ripe tomatoes inoculated with *Oidium lactis* (after 48 hours' growth in partially dry chamber, 18°–20° C.): *a*, in fresh slices; *b*, punctures; *c*, no inoculation but punctured.

FIG. 3.—Diseased ripe tomatoes spread open in moist chamber for 24 hours at 20° C.; white fungus prominent on open material.

FIG. 4.—Sliced ripe and green tomatoes inoculated with *Oidium lactis* (from 4 to 6 days' growth 18°–20° C. in partially dry chambers): *a*, on sliced ripe fruit; *b*, on green to half ripe fruit; *c*, no inoculation on sliced ripe tomato.

FIG. 5.—*Oidium* or *Oospora lactis:* *a* and *d*, mycelial branches and spores; *b*, spores of various sizes in chains; *c*, spoıes; *e*, spores in budding-like formation.



POOLE on FRUIT ROT

EFFECT OF SEEDS UPON HYDROGEN-ION CONCENTRATION OF SOLUTIONS[1]

W. RUDOLFS

In the course of a study on the effect of single salt solutions with definite osmotic concentration values upon absorption by seeds[2], it was found that the H-ion concentrations of the solutions in which the seeds were immersed changed markedly during the process of imbibition. The general interest and importance of this phenomenon in connection with seed studies made its further investigation highly desirable. Accordingly single salt solutions of magnesium sulphate, sodium nitrate, calcium nitrate, sodium chloride, potassium chloride, and potassium carbonate were prepared, ranging in osmotic concentration values from 0.001 of an atmosphere to 7.0 atmospheres. The seeds used in connection with these solutions were corn, spring wheat, white lupine, watermelon, Canada field peas, Japanese buckwheat, dwarf Essex rape, and alfalfa. Fifty seeds of each of the larger kind and 100 each of alfalfa and rape were placed in small bottles each containing 100 cc. of solution. The bottles were placed on a laboratory table, and the seeds were allowed to soak for a period of fifteen hours, after which the solutions were poured off and the H-ion concentrations determined by the colorimetric method, using the double tube standards of GILLESPIE[3] and the apparatus devised by VAN ALSTINE.[4]

The results of these determinations are given in tables I and II in terms of P_H values. The initial P_H values of the solutions are here compared with the final values determined at the end of

[1] Paper no. 83 of the Journal Series, New Jersey Agricultural Experiment Station, Department of Plant Physiology.

[2] RUDOLFS, W., Effect of salt solutions having definite osmotic concentration values upon absorption by seeds. Soil Science **11**:277–293. 1921.

[3] GILLESPIE, L. J., Colorimetric determination of hydrogen-ion concentration without buffer mixtures, with especial reference to soils. Soil Science **9**:115–136. 1920.

[4] VAN ALSTINE, E., The determination of hydrogen-ion concentration by the colorimetric method and an apparatus for rapid and accurate work. Soil Science **10**:467–478. 1920.

TABLE I

INITIAL AND FINAL P_H VALUES OF SINGLE SALT SOLUTIONS IN WHICH SEEDS WERE SOAKED FOR FIFTEEN HOURS

P_H values		Atmospheres											
		7	6	5	4	3	2	1	0.5	0.1	0.01	0.001	0.00
		$MgSo_4$											
Initial		6.5	6.5	6.4	6.4	6.3	6.3	6.2	6.2	6.1	6.0	6.0	6.0
After 15 hours	Corn	4.1	4.1	4.0	3.9	3.9	3.9	3.9	3.9	3.9	4.4	4.9	5.6
	Wheat	4.8	5.0	5.0	5.0	4.9	4.8	4.9	4.8	4.7	4.7	4.9	5.5
	Watermelon	4.9	5.0	5.0	5.0	5.0	5.0	5.0	4.9	4.8	5.1	5.8	5.8
	Peas	4.6	4.6	4.6	4.6	4.9	4.9	4.9	4.7	5.7	5.9	5.9	5.9
	Buckwheat	5.2	5.2	5.2	5.2	5.2	5.3	5.4	5.4	5.6	5.9	5.9	5.9
		$NaNo_3$											
Initial		6.6	6.5	6.5	6.4	6.4	6.3	6.2	6.1	6.0	5.9	5.9	5.9
After 15 hours	Lupine	5.7	5.7	5.7	5.6	5.7	5.7	5.7	5.7	5.8	5.7	5.7	5.9
	Rape	5.5	5.5	5.6	5.5	5.5	5.5	5.7	5.7	5.7	5.8	5.8	5.8
	Alfalfa	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.7	5.7	5.7	5.8
	Corn	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.3	4.7	4.9	5.2	5.5
		$Ca(No_3)_2$											
Initial		7.0	6.9	6.8	6.7	6.7	6.6	6.5	6.4	6.3	6.2	6.1	6.0
After 15 hours	Corn	4.2	4.2	4.2	4.2	4.1	4.1	4.1	4.1	4.5	5.5	5.5	5.9
	Lupine	5.5	5.5	5.6	5.6	5.6	4.9	4.9	4.8	5.2	5.6	5.5	5.8
	Peas	5.2	5.2	5.2	4.9	4.7	4.3	4.2	4.2	4.2	4.3	4.4	5.6
	Watermelon	4.2	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.2	4.9	5.1	6.0
		NaCl											
Initial		6.6	6.5	6.4	6.4	6.4	6.3	6.3	6.2	6.2	6.1	5.9	6.0
After 15 hours	Corn	4.1	4.2	4.1	4.1	4.1	4.2	4.2	4.3	4.7	5.1	5.1	5.7
	Wheat	4.9	5.1	5.1	5.1	5.0	5.1	5.0	4.9	4.9	4.9	5.3	5.9
	Rape	4.1	3.9	4.1	3.9	3.8	4.0	3.7	3.9	3.9	4.1	4.1	5.9
		KCl											
Initial		6.5	6.4	6.4	6.4	6.3	6.2	6.2	6.1	6.1	6.0	5.9	5.9
After 15 hours	Corn	4.1	4.1	4.1	4.1	4.1	4.1	4.2	4.3	4.7	5.0	5.1	5.9
	Wheat	5.9	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.6	5.8	5.8	5.8
	Lupine	5.7	5.7	5.6	5.7	5.7	5.7	5.8	5.9	5.9	5.9	5.9	5.9
	Peas	5.6	5.6	5.6	5.6	5.6	5.7	5.7	5.7	5.7	5.8	5.7	5.9
	Buckwheat	5.1	5.3	5.4	5.3	5.3	5.4	5.6	5.6	5.6	5.7	5.7	5.9
		K_2Co_3											
Initial		+*	+	+	+	+	+	+	+	+	8.4	5.9	5.9
After 15 hours	Lupine	7.5	7.5	7.5	7.5	7.5	6.7	6.7	6.7	5.5	5.9	5.9	5.9
	Corn	+	+	+	+	+	9.6	9.2	8.7	6.5	5.8	5.7	5.7
	Rape	+	+	+	9.4	8.8	8.2	7.5	7.5	6.2	5.7	5.5	5.9

* + = higher than 9.8.

definite time intervals, during which the seeds remained in contact with the solutions. Each of the data given represents the average of the results obtained from two or more trials made with the same number of seeds soaked in equal amounts of solution for time periods of the same duration.

From the data of table I it will be observed that in all cases the H-ion concentrations of the solutions were markedly increased by contact with the seeds, even when strongly alkaline solutions of potassium carbonate were used. The seeds immersed in solutions of different concentrations of a single salt had a tendency to bring the P_H values of the solutions to a point which was fairly constant for a given species of seed, regardless of the original salt concentration or of the initial P_H values of the solutions, except when the solutions were very dilute. The maximum reaction change produced by corn in the various concentrations of all the salts used, except potassium carbonate, brought the final P_H values of the solutions to approximately 4.1, varying only slightly above or below this value except in the very dilute solutions (0.01 and 0.001 atm.) as previously noted. While the final P_H values produced by the seeds of other species were approximately constant in the different concentrations of the same salt, they varied considerably in the solutions of the different salts. For example, the final P_H values produced by wheat in the magnesium sulphate solutions were around 4.9, in the potassium chloride solutions they were approximately 5.7. Watermelon seeds produced corresponding P_H values of approximately 5.0 in the magnesium sulphate solutions and 4.1 in calcium nitrate solutions. Seeds of other species produced similar differences in the P_H values of the solutions of the different salts.

It will be observed that the initial H-ion concentration of the solutions of each salt increased slightly with the progressive decrease in the total salt concentration, but the P_H values of the solutions after soaking the seeds in them for fifteen hours showed a striking similarity in value, except in the very dilute solutions. With a few exceptions the seeds were incapable of bringing about any marked reaction changes in the very dilute solutions (0.01–0.001 atm.), behaving in these solutions in somewhat the same manner with respect to changes in reaction as they did in distilled water.

The effect of the seeds upon the H-ion concentration of the solutions in which they were immersed is strikingly shown in the case of the potassium carbonate solutions. The P_H values of these solutions were well above 9.8. After fifteen hours' immersion,

TABLE II

SEEDS IMMERSED IN $MgSO_4$ FOR DIFFERENT PERIODS, RINSED IN DISTILLED WATER, AND THEN PLACED IN FRESH SOLUTIONS CORRESPONDING TO THE OLD

P_H values	Atmospheres											
	7	6	5	4	3	2	1	0.5	0.1	0.01	0.001	0.00
	Corn											
Initial	6.5	6.5	6.4	6.4	6.3	6.3	6.2	6.2	6.1	6.1	6.0	6.0
After 3 hours	4.1	4.1	4.2	4.2	4.1	4.1	4.1	4.1	4.0	4.1	4.9	5.9
After 5 hours	4.2	4.2	4.2	4.2	4.1	4.1	4.1	4.1	4.0	4.1	5.1	5.9
After 8 hours	4.1	4.1	4.1	4.1	4.1	4.0	3.9	3.9	3.9	4.0	4.9	5.9
After 15 hours	4.1	4.1	4.0	3.9	3.9	3.9	3.9	3.9	3.9	4.1	4.9	5.9
After 18 hours	4.1	4.1	4.0	3.9	3.9	3.9	3.9	3.8	4.1	4.2	5.0	5.7
After 21 hours	4.1	4.1	3.9	3.9	3.9	3.9	3.9	3.9	3.9	4.1	4.9	5.6
	Rinsed and placed in fresh solution											
Initial	6.6	6.5	6.5	6.4	6.4	6.3	6.2	6.2	6.1	6.1	6.0	6.0
After 1 hour	4.3	4.2	4.1	4.1	4.2	4.1	4.1	4.1	4.1	4.2	4.9	5.9
After 21 hours	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.2	4.9	5.7
After 40 hours	4.1	4.0	4.0	4.1	4.0	4.0	4.0	4.0	4.0	4.2	4.9	5.7
	Buckwheat											
Initial	6.5	6.5	6.4	6.4	6.4	6.3	6.3	6.2	6.1	6.1	6.0	6.0
After 15 hours	5.2	5.3	5.2	5.3	5.2	5.3	5.4	5.4	5.6	5.9	5.9	5.9
After 21 hours	5.2	4.9	4.9	4.9	4.7	4.9	4.9	4.9	5.1	5.3	5.5	5.7
After 40 hours	5.3	5.6	5.2	5.4	5.1	5.1	5.3	5.4	5.3	5.5	5.7	5.8
	Rinsed and placed in fresh solution											
Initial	6.6	6.5	6.5	6.4	6.4	6.3	6.2	6.2	6.1	6.1	6.0	6.0
After 1 hour	5.1	4.9	4.9	5.0	4.9	4.9	4.9	4.9	4.9	5.1	5.5	5.9
After 5 hours	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	5.1	5.1	5.5	5.9
After 15 hours	5.0	5.0	5.1	5.0	5.0	5.0	5.1	5.0	5.0	5.5	5.5	5.8

lupine seeds brought the P_H values of these strongly alkaline solutions below the neutral point in concentrations below three atmospheres, and to 7.5 in all the higher concentrations. Both corn and rape seeds immersed in the solutions of low concentration of this salt also increased the H-ion concentration to such an extent as to bring the P_H values below the neutral point.

The maximum reaction change which the seeds were capable of bringing about in the small quantities of solution here used (100 cc.) was accomplished in a comparatively brief period of time, as is shown by the data in table II. Fifty corn seeds or fifty buckwheat seeds immersed in 100 cc. of magnesium sulphate solution, with osmotic concentration values of 7.0 atmospheres or less, produced the maximum reaction changes of which they were capable during an interval of one hour or less. The rate of reaction change of course is determined by the amount of solution used, total salt concentration, the number of immersed seeds, temperature, etc.

After the maximum reaction changes which the seeds were capable of bringing about in the solutions had been attained, the P_H values then remained approximately constant for an indefinite period. When the seeds were removed from the old solutions, rinsed with distilled water for a few seconds, and placed in fresh solutions with corresponding concentrations of the same salt, the phenomenon of reaction change again took place and continued until the maximum H-ion concentration was the same as that previously produced. The final P_H values with corn in magnesium sulphate solutions were 3.9 to 4.2, except in the very dilute solutions, always varying slightly on account of differences in temperature and other environmental factors. The seeds may thus be immersed several times successively in fresh solutions, the reaction changes taking place each time, but always bringing the final P_H values to approximately the same point, which is fairly definite for each species in the solutions of a given salt, until finally the absorptive capacity of the seeds is exhausted, and equilibrium is established between seeds and solution.

The exact cause or causes of the rapid reaction change of the solutions as indicated by changes in the P_H values has not been determined with absolute certainty. There are without doubt several contributing factors, but all the experimental evidence thus far produced appears to indicate that the primary factor, and the only one which could account for the rapid reaction changes in the single salt solutions here used, is that directly related to ion absorption by the seeds, the H-ion concentration increasing as the cations are removed from solution by absorption at a more rapid rate

than the anions. This is in accord with the work of PANTANELLI,[5] who concludes from his extensive researches that salt intake by the cells of living plants is an absorption phenomenon of single ions, and he attributes the reaction changes of solutions in contact with the roots of green plants to the fact that some ions are absorbed at a more rapid rate than others.

That the materials excreted by the seeds in contact with the solutions here used can have little influence in bringing about the rapid increase in H-ion concentration, is indicated by the fact that seeds immersed in distilled water, under conditions similar to those under which they were immersed in the single salt solutions, did not bring about any marked reaction changes, even when the seeds were in contact with the solutions during a period of forty hours. This is shown by the data in the last columns of tables I and II.

The rates at which these reaction changes take place and the factors influencing them, together with a study of salt solutions of a wider range and of a number of mineral and organic acids will be the subject of a later report.

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N.J. AGRICULTURAL EXPERIMENT STATIONS
NEW BRUNSWICK, N.J.

[5] PANTANELLI, E., Über Ionenaufnahme. Pringsheim's Jahrb. Wiss. Bot. **56**: 689–733. 1915.

CURRENT LITERATURE

BOOK REVIEWS

Biology of aquatic plants

The writer of this interesting volume[1] has rendered a service to botanists in summarizing and bringing together the extensive but widely scattered work that has been done on aquatic seed plants. Mrs. ARBER'S work, however, is far more than a compilation, for every chapter contains interpretive portions, and in many of them the summary of the literature is but a scaffolding for further records or conclusions. Certain chapters are greatly enriched by the author's own experimental and philosophical conclusions.

Members of this biological group are of special interest because of their double specializations, first as terrestrial plants that have achieved flowers and seeds, and secondly because of the more or less marked modification of structure and reproductive method as now related to the aquatic environment. The volume will be of interest to all botanists, for the subject matter is ably grouped around fundamental biological topics, many of them most favorably illustrated by aquatic Angiosperms.

Part I deals with water plants as a whole, and takes up typical life histories, starting with a biological classification of hydrophytes in relation to water and substratum. The author then proceeds to discuss the vegetative structure of typical aquatics, including the marine Angiosperms, which are discussed in the concluding chapter of this section. In Part II the anatomy of leaves, stems, and roots is considered, together with modifications of the external form of these organs. The development of turions, or winter buds, is discussed, and the later portion of this section takes up flowers, pollination, fruits, and seed dispersal. Part III presents an analysis of the physiological conditions of the water habitat and the application of these to aquatic plants. This section is concluded by a chapter on the ecology of hydrophytes, which is conservatively handled, a topic which in the hands of some might perhaps have given title to the book. Part IV is concerned with philosophical discussions dealing with the dispersal and geographic distribution of water plants, their possible origin, and their relations to the theory of natural selection. The concluding chapter involves a summary of the author's work on the phyllode theory.

The volume is attractively printed and illustrated by 171 text figures, one-third of them original. The bibliography occupies seventy-two pages, and is made fully available by means of an appended index to the genera and

[1] ARBER, AGNES, Water plants, a study of aquatic Angiosperms. 8vo. pp. xvi+436. *figs. 171.* Cambridge University Press, England. 1920.

families of plants named in the literature. This is in addition to the general index for the volume as a whole.—R. B. WYLIE.

Chemistry of vegetable cell

A valuable service has been rendered to students of plant physiology by GRAFE,[2] who has prepared a textbook dealing with the biophysics and biochemistry of the processes of cell life in plants. The work was ready for publication when the war broke out, and has been delayed almost eight years by the conditions in Europe. This does not mean that the text is already old, for the delay has permitted a revision since the war, and much new material has been incorporated, particularly that which has been the result of discoveries in the laboratories of Europe. Any failure to note foreign results is excused by GRAFE on the grounds of the great difficulty experienced in keeping in touch with the scientific production of other nations.

The subject is presented under five main headings: the physicochemical laws of cell phenomena; light and heat as energy factors; the cell wall; the protoplasm; and dynamic chemistry. The first two sections occupy about a fourth of the text, and survey the problems of diffusion and osmosis, colloidal state, imbibition, adsorption, electrolytic dissociation, energy transformations, and catalysis. The third section is the briefest one, and deals with the structure, composition, and chemical transformations and modifications which occur in the walls of cells during development and maturation.

The section on protoplasm considers its colloidal structure, chemical constitution, the enzymes, toxins, precipitins, and pigments of the cell, particularly the chlorophyll and anthocyanin pigments. The final section is the longest and most important one, and considers the chemical transformations of cell activity. The author reverses the usual order of presentation by considering the utilization of energy and the respiratory processes first, then a brief consideration of stimulus and response, closing with the constructive energy-storing processes.

The book is intended as a general text for students, and the author has written in a style that is commendable for its clarity and directness. The literature list at the end of the volume occupies twenty double column pages, and the total number of references is nearly a thousand. Some of these references are not considered in the text of the volume, but are included for those who desire to orient themselves with reference to the literature on cell chemistry.—CHAS. A. SHULL.

Poisonous plants

A textbook dealing with the poisonous plants and weed seeds of Canada and the Northern United States has been prepared by THOMSON and SIFTON.[3]

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[2] GRAFE, VICTOR, Chemie der Pflanzenzelle. 8vo. pp. viii+420. *figs. 32.* Berlin: Borntraeger. 1922.

[3] THOMSON, R. B., and SIFTON, H. B., A guide to the poisonous plants and weed seeds of Canada and the northern United States. 8vo. pp. 169. *figs. 40.* University of Toronto Press. 1922.

It is intended to meet the needs of the veterinarian, the farmer, the stockman, and to a more limited extent the physician and the general public. The high standing of the authors and the fact that they are botanists insure accuracy so far as the plants are concerned.

The book is divided into four sections, the first three dealing with the plants mainly responsible for fatalities among animals, grouped on the basis of their occurrence in the animal's feed, whether found in hay (section 1), in pasture (section 2), or in concentrated feed stuffs (section 3). The fourth section deals with the plants which, although poisonous, seldom cause the death of animals. The larger number of poisonous plants, including those mainly responsible for poisoning in man, belong here. The forty illustrations are original and will enable the amateur to identify the plant responsible in an ordinary case of poisoning. There is a "symptoms" key, not claiming to be precise, but which will facilitate diagnoses by suggesting the plants which should be looked for when certain symptoms are observed and plant poisoning is suspected.

The authors emphasize the need for research on poisonous plants, both along the lines of chemical analysis and feeding experiments. The book is interesting and well written, and many of the observations, which could have been made only by experienced botanists, indicate the propriety of issuing such a book from a botanical department.—C. J. CHAMBERLAIN.

Constitution of vascular plants

CHAUVEAUD,[4] in a small volume, has presented his views as to the constitution of vascular plants, based upon an investigation of ontogeny. He calls attention to the disagreement between current theories and the facts he has observed in his investigations. His conclusion is that the body of vascular plants is built up by the successive appearance of fundamental units, or "phyllorhizes." As one passes from Pteridophytes to Spermatophytes, the disappearance of the root element ("rhize") is more and more frequent, and becomes the rule in Dicotyledons, where a rhize appears in connection with the first two phyllorhizes, but appears in connection with later phyllorhizes only in exceptional cases, as an adventitious root. In consequence of being thus reduced to a single member, the root has acquired the power of enlarging indefinitely and of persisting as long as the stem itself.

It is shown, also, that in the development of the apparatus of conduction there is a complete parallelism between internal and external morphology, since the vascular apparatus of the plant is built up by the repeated formation of elementary systems, each one corresponding to one of the phyllorhizes. Each bud is the beginning of a new phyllorhize to be added to the phyllorhize complex. In short, plants with roots are formed from elementary plantules or phyllorhizes, and the constitution of these is the unit of morphology.—J. M. C.

[4] CHAUVEAUD, GUSTAVE, La constitution des plantes vasculaires révélée par leur ontogénie. 8vo. pp. 155. *figs.* 54. Paris: Payot. 1921. 10 fr.

NOTES FOR STUDENTS

Taxonomic notes.—LINDAU[5] has published the following new genera of Acanthaceae from Colombia: *Syringidium* and *Kalbreyeriella*.

NAKAI[6] has described eighteen new Japanese species of *Viola*.

HITCHCOCK[7] has published an account of the grasses of British Guiana based upon his collection during the autumn of 1919, and also upon the collection of the Jenman Herbarium. The list of grasses thus far includes 169 species, ten of which are described as new, four of them being species of *Panicum*. Most of British Guiana is covered by virgin forest, with extensive upland savannas only in the south. Of the 169 species included, thirty-three are introduced, the greater proportion of these being in the vicinity of settlements.

DIXON[8] has published an account of the mosses of the Wollaston Expedition to Dutch New Guiana in 1912–1913. He states that "the moss flora of New Guiana affords, perhaps, the most interesting field for the present day bryologist. Large tracts of Central Africa remain no doubt comparatively unexplored, and recent discoveries there show that much is still to be expected of bryological interest." The report includes also some additional mosses from British New Guiana. Among the species enumerated, twenty-three are described as new.

COMPTON[9] has published an account of the plants collected in New Caledonia and the Isle of Pines in 1914. The Gnetales and Ginkgoales are absent from New Caledonia, and the Cycadales are represented by a single species, which occurs only in the littoral zone. On the other hand, the Coniferales are developed to quite an exceptional degree, being represented by thirty-one species, an unusually large number for so small an area. Another remarkable feature of the coniferous flora is that apparently the whole of it is endemic. The range of the Taxineae is extended to New Caledonia by the new genus *Austrotaxus*, while the Cupressineae include the new genus *Callitropsis*.

DIXON[10] has described the following new genera of mosses, mostly from Africa: *Nanobryum*, *Chionoloma*, *Beddomiella*, *Œdipodiella*, *Chamaebryum*, *Physcomitrellopsis*, and *Dimorphocladon*.

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[5] LINDAU, G., Neue Gattungen der Acanthaceen. Notizblatt Bot. Gart. Berlin-Dahlem **8**: 142–144. 1922.

[6] NAKAI, TAKENOSHIN, Violae novae Japonicae. Bot. Mag. Tokyo **36**: 29–39. 1922.

[7] HITCHCOCK, A. S., Grasses of British Guiana. Contrib. U.S. Nat. Herb. **22**: 439–514. *figs. 77–86*. 1922.

[8] DIXON, H. N., The mosses of the Wollaston Expedition to Dutch New Guiana. Jour. Linn. Soc. **45**: 477–510. *pls. 28, 29*. 1922.

[9] COMPTON, R. H., A systematic account of the plants collected in New Caledonia and the Isle of Pines by R. H. COMPTON in 1914. Part II. Gymnosperms. Jour. Linn. Soc. **45**: 421–434. *pls. 26, 27*. 1922.

[10] DIXON, H. H., Some new genera of mosses. Jour. Botany **60**: 101–110. *pl. 564*. 1922.

DIELS,[11] in publishing an account of the Myrtaceae of Papua, describes numerous new species, and establishes the following new genera: *Xanthomyrtus* (14 species) and *Octomyrtus* (3 species). The large genera are *Jambosa* (50 species), and *Syzygium* (44 species).

GANDOGER[12] has published the first part of a series of descriptions of new species from various countries of the world. This first paper includes descriptions of 348 new species, a very large contribution to taxonomy.

SMITH and EVANS[13] have described a new genus (*Craigia*) of Sterculiaceae from China, province of Yunnan. It is a tree or shrub, and in foliage and inflorescence suggests certain species of *Sterculia*.

SARGENT[14] has described twelve new species of *Crataegus*, chiefly from Missouri and Arkansas.

BLAKE[15] has described forty-six new species of plants from Guatemala and Honduras, from a collection made during 1919 by members of an Economic Survey Mission sent out by the State Department. The new species are distributed among twenty-three families, and include two new genera: *Decazyx* (Rutaceae) and *Prosanerpis* (Melastomaceae).

ROBINSON,[16] in his further study of the Eupatorieae, has published seventeen new species of *Mikania* and one new species of *Ageratum*. He has also published [17] local revisions of *Mikania* covering certain regions of South America, as follows: Colombia (32 spp.), Venezuela (13 spp.), Ecuador (18 spp.), Peru (37 spp.), and Bolivia (28 spp.).

NAKAI,[18] in continuation of the study of the woody flora of Korea, has published an elaborate account of the Caprifoliaceae, accompanied by numerous unusually fine plates. Thirty-six species are recognized, distributed among six genera, much the largest being *Lonicera*, with seventeen species. The descriptions and discussions are in both Japanese and English, so that the publication is available for all taxonomists.

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[11] DIELS, L., Die Myrtaceen von Papuasien. Engler's Bot. Jahrb. **57**:356–400. 1922.

[12] GANDOGER, M. MICHEL, Sertum plantarum novarum. Pars prima. Bull. Soc. Bot. France. **65**:24–69. 1918.

[13] SMITH, W. W., and EVANS, W. EDGAR, *Craigia*, a new genus of Sterculiaceae. Trans. Bot. Soc. Edinburgh **28**:69–71. *pl. 1*. 1921.

[14] SARGENT, C. S., Notes on North American trees. IX. Jour. Arnold Arboretum **3**:1–11. 1921.

[15] BLAKE, S. F., New plants from Guatemala and Honduras. Contrib. U.S. Nat. Herb. **24**:1–32. 1922.

[16] ROBINSON, B. L., Records preliminary to a general treatment of the Eupatorieae. I. Contrib. Gray Herb. N.S. no. 64. pp. 1–21. 1922.

[17] ———, The Mikanias of northern and western South America. *Idem*. no. 64. pp. 21–116. 1922.

[18] NAKAI, TAKENOSHIN, Flora sylvatica Koreana. XI. Caprifoliaceae, pp. 92. *pls. 42*. Seoul. 1921.

PAYSON[19] has published a monograph of the genus *Lesquerella*, recognizing fifty-two species, only two of which are new. The synonymy and specimens examined are recorded in detail. Preceding the taxonomic presentation, there is an interesting discussion of the general morphology, phylogeny, and geographical distribution of the genus.

PETCH,[20] in continuation of his studies of entomogenous fungi, has presented a very detailed account of *Hypocrella* and *Aschersonia*. In *Hypocrella* twenty-nine species are described, two of which are new; while in *Aschersonia* thirteen species are described, four of which are new. In addition to the species included in the systematic presentation, a number of species are named as not seen, doubtful, or excluded.—J. M. C.

Origin of variations.—Of extreme interest to students of genetics is a recent number of the *American Naturalist* which contains the papers presented at the Toronto meetings in a symposium on "The origin of variations." JENNINGS,[21] discussing "variation in uniparental reproduction," stresses the fact that the vast majority of observed variations in primitive organisms are strictly non-heritable. There exists, however, a very small residuum of heritable variations, and in considering their evolutionary significance, two types should be distinguished. Seemingly spontaneous changes (mutations ?) appear to have occurred in some forms during a series of asexual generations, and have been isolated by selection to produce new constant races. The cause of these changes is unknown. On the other hand, variations have been induced by environmental changes, which have always reverted to the normal type after a certain number of asexual generations. JENNINGS points out that the period of persistence of such variations evidently depends, in good part, on the number of generations through which the producing agent acted, and expresses the belief that heritable characters, as permanent as any that are known to exist, might be produced by allowing the producing agent to act over a sufficient period of time.

BLAKESLEE[22] describes his work on *Datura*, which by this time has become well known,[23] showing how striking heritable variations accompany changes in chromosome number. These changes in chromosome number may result either from non-disjunction of one or a few chromosome sets, producing "unbalanced types," or may involve a wholesale doubling of all the chromosome sets, giving

[19] PAYSON, E. B., A monograph of the genus *Lesquerella*. Ann. Mo. Bot. Gard. **8**: 103–236. *figs. 34*. 1921.

[20] PETCH, T., Studies in entomogenous fungi. II. The genera *Hypocrella* and *Aschersonia*. Ann. Roy. Bot. Gard. Peradeniya **7**: 167–278. *pls. 2–5*. 1921.

[21] JENNINGS, H. S., Variations in uniparental reproduction. Amer. Nat. **56**: 5–15. 1922.

[22] BLAKESLEE, A. F., Variations in *Datura* due to changes in chromosome number. Amer. Nat. **56**: 16–31. 1922.

[23] BOT. GAZ. **72**: 178–182. 1921.

the "balanced" tetraploid (or indirectly, triploid) types. The tetraploid types breed true, but the others produce (in various proportions) several types of progeny, including individuals like themselves and others like the "normal" (pure diploid) original ancestors.

MULLER[24] takes up changes in the individual gene ("locus changes" or true mutations), and discusses their general characteristics. It is important to realize that the change is not always a mere loss, for clear-cut reverse mutations have been obtained in corn, *Drosophila*, and *Portulaca*. If the original mutation was a loss, the reverse mutation must be a gain. "It is generally true that mutations are much more apt to cause an apparent loss in character than a gain, but the obvious explanation for that is, not because the gene tends to lose something, but because most characters require for proper development a nicely adjusted train of processes, and so any change in the genes, no matter whether loss or gain, substitution or arrangement, is more likely to throw the developmental mechanism out of gear, and give a 'weaker' result, than to intensify it." MULLER depicts a very interesting and suggestive analogy between the gene and certain immunity reactions.

BRIDGES[25] elucidates the following very significant thesis. The characters of an organism, instead of being absolutely "determined" by a single gene, should rather be thought of as being acted upon simultaneously by many genes. Some genes tend to make a character more pronounced, and others to make it less pronounced, so that the grade of development actually realized by each particular character will be determined by the equilibrium between its modifying genes. The justification for this thesis appears from a consideration of some of BRIDGES' non-disjunctional *Drosophilas*, which exhibit previously unknown grades in the expression of a number of characters. Most startling are the cases where the character involved is sex itself; so that the fruitfly, previously the best known example of qualitative differentiation of sex on the basis of the X and Y chromosomes, now provides the most promising example of a quantitative sex mechanism with the newly discovered "intersexes" and "supersexes."[26]

EMERSON[27] presents and classifies a great mass of evidence on bud variation. He considers separately "somatic mutation of genes" and "somatic segregation," and under the latter heading "chromosome elimination," "cytoplasmic segregation," and "graft hybrids and other chimeras." This article should be of unusual interest to botanists.

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[24] MULLER, H. J., Variations due to change in the individual gene. Amer. Nat. **56**:32–50. 1922

[25] BRIDGES, CALVIN B., The origin of variations in sexual and sex-limited characters. Amer. Nat. **56**:51–63. *figs.* 7. 1922.

[26] BOT. GAZ. **72**:408–410. 1921.

[27] EMERSON, R. A., The nature of bud variations as indicated by their mode of inheritance. Amer. Nat. **56**:64–79. 1922.

GUYER[28] draws the following conclusions from his own well known experiments with white rabbits, and from the results of other investigations. Basically inheritance is mainly a question of the perpetuation of specific protein-complexes, and development the result of differential reactions of these same fundamental constituents under differing conditions of environment. There is evidently some degree of constitutional identity, probably protein homology, between the nature substance of a tissue and its correlative in the germ. Changes which can affect certain constituents of tissue cells initiate an influence which, borne in the circulating fluids of the body, evidently is able to affect the homologous constituents of the germ cells. This, of course, furnishes the basis for a Lamarckian view. The author feels that here may be a basis for progressive evolution.—M. C. COULTER.

Influence of host on parasite.—Continuing his studies on the physiology of parasitism, BROWN[29] has investigated the exosmose of substances from leaf and petal surfaces of several flowering plants, and the influence of such substances on the behavior of *Botrytis* spores. Drops of distilled water uniform in size were placed for twenty-four hours on petals of *Cereus*, *Phyllocactus*, *Gloxinia*, *Lilium*, *Papaver*, *Iris*, *Petunia*, *Tulipa*, *Rosa*, *Begonia*, *Viola*, *Lathyrus* (sweet pea), *Dahlia*, *Geranium*, *Cydonia*, *Pyrus*, and on leaves of several plants, including the broad bean. The change in the drops due to exosmose was determined by studying their capacity for germinating spores added to the drops in water suspensions, and also by electrical conductivity tests. Capacity for germinating spores was based on the average length of the germ tubes. An increase in conductivity resulted in all cases, accompanied in the greater number of plants studied by increased germination capacity, when the drops subjected to exosmose were compared with drops of distilled water of similar size. Petals difficult to wet gave lower conductivity and germination figures. In some plants, with leaves of *Tradescantia discolor*, for instance, increased conductivity was accompanied by germination capacity only equal to or less than that of distilled water, or by actual inhibition of germination. The exact source and nature of the inhibiting substance were not determined.

Attention is directed to the difference in the behavior of fungal parasites. Some, like the rust fungi, penetrate both susceptible and immune varieties of plants, their fate thereafter being determined by internal conditions. Contrasted with this is the behavior of *Botrytis* spores on the leaf of the broad bean, typical of another category of fungal parasites, in which the germination and attack depend upon the exosmose of substances into the infection drop, which can be used as a nutrient by the fungus.—J. G. BROWN.

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[28] GUYER, M. F., Serological reactions as a probable cause of variation. Amer. Nat. **56**:80–96. 1922.

[29] BROWN, WILLIAM, Studies in the physiology of parasitism. Ann. Botany **36**: 101–119. 1922.

Calamites.—Impressions of casts of the external features of *Calamites* stems are still very little known in comparison with the pith casts of the same plants. In all four of the large monographs now available on the *Calamites*, by STUR, WEISS, KIDSTON, and especially JONGMANS, by far the greater number of the figures relate to the pith casts. As a rule, examples of both medullary casts and impressions showing the true external features of the stem are mixed together in confusion, and are all referred to a common genus, *Calamites*. Neither from the generic nor the specific names employed can one distinguish whether one is dealing with pith casts or with the rarer external surfaces of these stems. It is hardly necessary, however, to point out that incrustations of the external features of the stems of these plants are of an entirely different morphological nature from the medullary casts. For this reason ARBER had proposed in 1916 a new form genus, *Calamophloios*, for the external stem impressions, with the exception of the very distinct type of *Dictyocalamites* which had been established already in 1912.

ARBER and LAWFIELD[30] intend to establish a number of species of these two new types, and they suggest that the same specific name may be used for both types of preservations, the internal and external ones. They avoided adopting new specific names for the types of *Calamophloios*, therefore, as compared with the pith casts. The authors also succeeded in carrying out a correlation of a number of external surfaces and pith casts of *Calamites*.—A. C. NOÉ.

Pliocene flora of Varennes.—The Pliocene flora of Varennes is the subject of a monograph by DE LA VAULX and MARTY,[31] which is divided into the following sections: (1) geology of the fossiliferous beds of Varennes, (2) a critical study of the fossil plants of Varennes, (3) a description of new species discovered in the deposits of Varennes, (4) paleontological, stratigraphical, botanical, and climatological conclusions; also a chapter on fossil Diatomaceae of Varennes is added. Of the forty-seven genera which are described in the fossil flora of Varennes, and which belong to twenty-three families, thirty-eight specific determinations could be made. Fourteen new species were established.

Ecologically, the flora of Varennes indicates a temperate climate, because of the thirty-seven definite species found at Varennes, thirty-four belong to the temperate zone. This flora also contains more continental than insular species. Almost all the species which form the flora of Varennes, or their nearest living relatives, inhabit at present the more or less mountainous districts

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[30] ARBER, E. A. NEWELL, and LAWFIELD, F. W., On the external morphology of the stems of *Calamites*, with a revision of the British species of *Calamophloios* and *Dictyocalamites* of Upper Carboniferous age. Jour. Linn. Soc. **44**:507–530. *pls. 23–25.* 1920.

[31] DE LA VAULX, ROLAND, and MARTY, PIERRE, Nouvelles recherches sur la flore fossile des environs de Varennes. Rev. Gen. Bot. **32**:282–300; 327–336; 351–68. 1920.

of southern Europe, Asia Minor, Japan, and the central states of the United States. This zone follows approximately the fortieth degree of northern latitude, and indicates an annual average temperature of 12–14°C. Since the Miopliocene, during which the fossil plants of Varennes lived, the most of its components have emigrated toward the south. The plant deposits of Varennes accumulated in a lake, into which the ashes of a volcano fell.—A. C. NOÉ.

Fossil plants from Missoula region.—A paper by JENNINGS[32] deals with some fossil plants from beds which are believed to be of Oligocene age. The fossil plants consist of impressions of leaves and of leafy twigs, there being also some impressions of fruits and leafless twigs. The Missoula specimens are embedded in fine-grained volcanic ash which preserved the finer venation of the leaf surfaces. Twenty-one species are enumerated, ten of which are described as new, and one of which required a new name. Of the fifteen genera represented in the Missoula flora, all but two are also represented in the Florissant Basin of Colorado. The Missoula flora probably occupied the shores and surrounding slopes of a high mountain lake. The climate was warmer, and probably drier than that now prevailing at recent localities of similar geographic position, like, for instance, the Flat Head Valley; and the vegetation represented by the Missoula fossils ranged probably throughout a series of associations from wet meadow to moderately xerophytic oak forests on rocky or sandy shores. All of these vegetational associations were in close proximity to the waters of a lake. There are eleven plates with excellent illustrations in the book.—A. C. NOÉ.

Cycadofilicales.—CARPENTIER[33] presents a most interesting paper on a series of Cycadofilicales fructifications which were from the Lower Carboniferous of northwestern France. Two genera of seeds (*Lagenospermum* and *Carpolithus*) have been observed, and in a number of instances pictured also. Sporangia or microsporangia of *Telangium*, *Pterispermotheca*, and *Diplotheca* are described. CARPENTIER concludes that the small seeds of *Lagenospermum* and of related genera seem to have belonged to *Sphenopteris*, probably *S. Hoeninghansi* and *S. elegans*. While the occurrence of *Sphenopteris* together with seeds of *Lagenospermum* is frequent in the Westphalian of northern France, the seeds of *Neuropterides*, which occur frequently in the Bassin de Valenciennes, are very rare in the Bassin de la Basse-Loire. CARPENTIER also emphasizes that our knowledge of the microsporangia of the Cycadofilicales of Mouzeil and the Bassin de la Basse-Loire is still very rudimentary, only fragments of male inflorescences having been discovered. They seem to have been of a very delicate structure. *Telangium*, or a nearly related genus,

[32] JENNINGS, O. E., Fossil plants from the beds of volcanic ash near Missoula, Western Montana. Mem. Carnegie Museum **8**:385–427. *pls. 22–23*. 1920.

[33] CARPENTIER, A., Contribution à l'étude des fructifications du Culm de Mouzeil (Loire-inférieure). Rev. Gen. Bot. **32**:337–349. 1920.

originated in the Devonian and flourished in the lower and upper Culm in Basse-Loire and during the Westphalian in the north.—A. C. NOÉ.

Availability of potassium.—BREAZEALE and BRIGGS[34] find that the potassium of orthoclase solutions is not available for wheat seedlings, owing, it is concluded, to the potassium being present with other elements in a complex solute molecule, which does not yield potassium ions. This conclusion is supported by the fact that oxidation with acids makes the potassium available. From the experiments recorded in the paper, the general conclusions are drawn that the concentration of a plant food in the soil solution is not necessarily a measure of its availability for the plant, and that applying finely ground orthoclase to a soil does not immediately increase the available potash content of the soil. While the conclusions are probably justified, it must not be concluded from experiments of this kind that plants cannot get the needed potassium from finely ground orthoclase applied to the soil or from orthoclase found naturally in the soil. The nature of the root system and the conditions of its functioning are probably quite different in the solution than in the soil.—S. V. EATON.

Indian Gondwana plants.—A great majority of the specimens described in this volume were figured by FEISTMANTEL in the *Palaeontologia Indica*. A revision[35] of the material brought to light some new features, and in several instances has revealed inaccuracies in the illustrations accompanying FEISTMANTEL'S descriptions. Numerous text illustrations and seven plates in folio with excellent drawings and photographs enable the reader to judge SEWARD'S revision of Gondwana plants. SEWARD was ably assisted by SAHNI, who promises to become an authority on Indian paleobotany.

The Gondwana system is an extremely interesting geologic period of high paleobotanic importance. It corresponded to the Permo-Carboniferous of Europe, and is distinguished by paleozoic glaciation features. The Gondwana flora is characterized by a wealth in gymnosperms, especially Cycadophyta. The present volume describes eight species of Bennettitales, and seven species of Nilssoniales; also numerous Cordaitales, Ginkgoales, and Coniferales are represented, but the pteridophytes are rather scarce. No *Glossopteris* is mentioned.—A. C. NOÉ.

New method of vegetative multiplication.—DASTUR and SAXTON[36] have described a method of vegetative multiplication in a perennial species of

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[34] BREAZEALE, J. F., and BRIGGS, L. J., Concentration of potassium in orthoclase solutions not a measure of its availability to wheat seedlings. Jour. Agric. Res. **20**:615–621. 1921.

[35] SEWARD, A. C., and SAHNI, B., Indian Gondwana plants: A revision of Palaeontologia Indica. New Series **7**:1–42. *pls. 1–7*. 1920.

[36] DASTUR, R. H., and SAXTON, W. T., A new method of vegetative multiplication in *Crotalaria burhia*. New Phytol. **20**:228–233. *figs. 4*. 1921.

Crotalaria (*C. burhria*) which differs from anything previously described. The plant has a very long tap root, and when about a year old the axis becomes ribbed, the ribs beginning at the transition region between stem and root and extending in both directions. The ribbing is associated with the development of an accessory bundle system, and the gradual separation of branches which become established as separate plants. In this way, "when the main axis perishes, a circle of branches separated to below the ground level is already established." It was also observed that although the plant flowers during most of the year, it seems seldom to develop seeds.—J. M. C.

Sexual evolution.—SCHAFFNER[37] has presented his conception of the evolutionary stages of sexual expression, defining what may be called twenty-three steps in evolutionary progress, each one illustrated by examples. He is convinced that sex "cannot be associated primarily with special chromosomes." The general conclusion is reached that "the specific structures and functions developed in the ontogeny of an organism appear to be conditioned on the interaction of four fundamental influences: (1) hereditary factors, (2) influence of environment, (3) progression of senility, and (4) presence of sexual states in the living substance."—J. M. C.

Mesozoic flora.—BERRY'S[38] fourteenth contribution to the Mesozoic flora of the Atlantic coastal plain deals with the floras of the Eutaw and Ripley formations. The article comprises an advance paper of the fuller material to be described and illustrated in a professional paper of the United States Survey (no. 112) which has meanwhile appeared. The larger publication includes the Tuscaloosa formation besides the two groups mentioned.—A. C. NOÉ.

North American flora.—Part I of volume 6 begins the presentation of Phyllostictales by SEAVER. This ordinal name is used in place of Sphaeropsidales, because the generic name *Sphaeropsis* "goes out of the order," and the ordinal name becomes untenable. In this first part the genus *Phyllosticta* is presented, 300 species being recognized, only three of which are described as new.—J. M. C.

Fossil woods of Queensland.—SAHNI[39] describes and gives microphotographs of a number of fossil woods which range from fern stems through gymnosperms to angiosperms. The paper is a valuable contribution to the study of Mesozoic woods.—A. C. NOÉ.

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[37] SCHAFFNER, J. H., Progression of sexual evolution in the plant kingdom. Ohio Jour. Sci. **22**:101–113. 1922.

[38] BERRY, E. W., Contributions to the Mesozoic flora of the Atlantic coastal plain. XIV. Tennessee. Bull. Torr. Bot. Club **48**:55–72. 1921.

[39] SAHNI, BIRBAL, Petrified plant remains from the Queensland Mesozoic and Tertiary formations. Queensland Geol. Survey. Publ. no. 267. pp. 48. *pls. 5. figs 10.* 1920.

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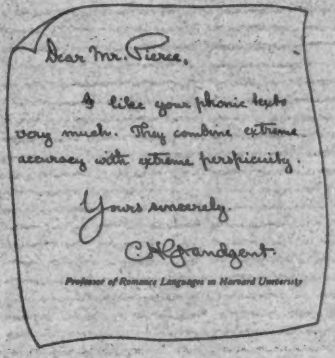
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